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Award Number: W81XWH-10-2-0140

TITLE: Development of a Novel Translational Model of Vibration Injury to the Spine to Study Acute Injury in Vivo

PRINCIPAL INVESTIGATOR: Beth A. Winkelstein, Ph.D.

CONTRACTING ORGANIZATION: University of Pennsylvania,
Philadelphia, PA, 19104-6205

REPORT DATE: October 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01- Oct-2012	Annual	30 Sep 2011 – 29 Sep 2012

4. TITLE AND SUBTITLE
Development of a Novel Translational Model of Vibration Injury To the Spine to Study Acute Injury in Vivo

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0140

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Beth A. Winkelstein

winkelst@seas.upenn.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pennsylvania
Philadelphia, PA 19104-6205

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
And Material Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

There is currently little mechanistic data defining the relationship between whole body or spine vibration, physiology and pain. Considering that pain is tremendous problem, we have developed a novel in vivo model to study how vibration produces chronic pain and the associated effects on tissues and pain cascades. Studies to date have revealed that even 30 minutes of daily vibration for only 7 days is sufficient to induce significant widespread pain that is sustained following the termination of vibration. Moreover, a single day of exposure also induces sensitivity but it is only transient and recovers by 7 days; yet, if a second exposure is introduced after that recovery, the time to recovery is lengthened, suggesting that there is a cumulative effect of even those exposures that might be considered benign. Another finding is that a host of biochemical changes are associated with pain, including modifications in the cellular response of muscle, spinal cord and intervertebral discs. Analysis of transmissibility demonstrates the resonant frequency of the rat spine to be 8 Hz, while analysis of human studies indicates the response of that species is 4 Hz. Together, all of these findings have tremendous implications for both sub-failure spinal injury and pain. They also establish a strong foundation for the remaining studies including additional exposures and defining the time course of the onset and/or resolution of the physiological responses. Continued investigations in these areas, as well as in the mathematical models we have begun to establish will integrate findings across all tasks of this work.

15. SUBJECT TERMS
Pain, vibration, spine, transmissibility, biomechanics, injury

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	46	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

This project combines military injury expertise with pain modeling to develop *in vivo* rat models of painful injury mimicking military injuries, in order to serve as a platform system to understand injury risk, biomechanical mechanisms of painful injury, and to evaluate measures for injury prevention and treatment. In particular, this project is focused on whole body vibration along the spine's axis and a single jolt loading. There are three coordinated major activities under this project to ensure we successfully achieve our goals to: (1) identify those schedules of whole body vibration that present the greatest risk for inducing tissue injury, pain and/or spinal instability, (2) develop a useful animal model to study these injuries, and (3) establish risk evaluation criteria to identify which injuries and exposures are most threatening. This research project utilizes biomechanical, *in vivo* and biochemical approaches to define injury and pain mechanisms by which vibration and/or jolt initiates a pain response – either acute or crhonic. We proposed an interdisciplinary research approach between collaborators at an academic research institution and the USAARL, in order to develop effective methods to study the most-relevant injuries and to develop a relevant *in vivo* model system would provide such a tool.

In the last year of this project we have made good progress on the development of several different models of whole body vibration that induce sustained and transient pain, separately. We have met the associated timeline of activities and milestones that were laid out in the approved statement of work for this effort. We have also completed critical studies to define the anatomic and mechanical scaling differences between the rat and the human and are implementing them together with ongoing analysis of human data to develop appropriate and meaningful algorithms for evaluating risk for injury as this project moves forward. Lastly, we have initiated studies to generate tissues to define the temporal development of inflammatory, nociceptive and injury responses. Through such assays of spinal columns and spinal cords in the pain-producing scenarios we have uncovered novel relationships between tissue loading, changes in the intertervertebral disc and interesting cascades in the spinal cord, that may contribute to chronic pain in our model. With another productive year of this project we are also poised now to carry out the next set of investigations that more-deeply investigate the whole body mechanics in our rat model, the human kinematic and kinetic response, and that utilize different exposures, such as jolt, along with the ongoing execution of studies to define the temporal tissue responses that will help shape our mechanistic understanding of the pathophysiology of pain from vibration.

BODY

Over the past year of the project, we have made progress on all of the Tasks that were originally proposed to occur during the second year of our project. Having received approval for the use of human data in our analysis for Task 2, we spent several months this past year obtaining and organizing those data to make progress in the previously delayed Task 2. We have continued to integrate the *in vivo* studies with that work as well and presented findings in 6 presentations at national meetings in the last year, and have submitted an additional abstract and are working on 3 papers that will be submitted in the next few months.

In this portion of the report we summarize activities related to those publications and refer to the full-publications (provided in the Appendix), as well as report on the methods and results for the additional studies in detail. A primary goal of this work is to develop *in vivo* rat models of painful injury from vibration and jolt that mimic real-world injuries, in order to serve as a platform system to understand injury risk and biomechanical and biochemical injury mechanisms. Since our last report, the majority of the work has been focused on completing the anatomic and biomechanical scaling studies, developing a simple mechanical mathematical model to help understand and model our *in vivo* studies, the refinement of the vibration device and data acquisition system, and performing the *in vivo* studies to determine the different exposure profiles. We structure this section of the report to provide an overall

summary of each Task and its related status, followed by a more-detailed report of the data and findings for each Task.

The GANTT chart below summarizes the timing of the specific tasks that are associated with each aim across the entire project period under the approved statement of work. Before providing a detailed record of the research findings in this period, we indicate the current status of each activity in that chart to provide an overview of the research activities that were completed in the previous report, completed during this most recent period, and that are *ongoing* and *planned*.

The majority of activities originally proposed to occur in Year 2 involved performing the review of data from the field and MARS simulations and revising exposures as needed under Aim 1 (Task 2). Due to the delay in obtaining approval, some of these efforts are still ongoing. However, as previously reported, we had initiated selected activities under Aims 3 and 4 in Year 1 and so have completed the anatomical scaling studies and have established the loading conditions for the *in vivo* studies of whole body vibration. Accordingly, device modifications and in vivo studies with jolt are ongoing and planned for the early part of Year 3. Lastly, we have initiated some of the time point studies for temporal characterization of tissue responses (Aim 3) and actively revising drafts of manuscripts for publication (Task 5). We provide detailed explanation of these and all Tasks in the following detailed summary broken down for each Task.

TASK	Year 1	Year 2	Year 3	Year 4
TASK 1 – Obtain Regulatory Approvals (Year 1)				
1a. Obtain regulatory approval for animal studies	completed			
1b. Obtain regulatory approval for use of human data	Completed			
TASK 2 – Aim 1: Review of Injury Exposures in Theater (Years 1 & 2)				
2a. Review field data	*Ongoing*			
2b. Review MARS data	*Ongoing*			
2c. MARS simulations		*planned*		
2d. Revise exposures		*planned*		
2e. Publish findings	*planned*			
TASK 3 – Aim 2: Design Experimental System & Perform Scaled Loading Studies (Years 1-3)				
3a. Design initial injury device	completed			
3b. Perform scaling studies	Completed			
3c. Perform pilot studies with injury device	Completed			
3d. Modify/redesign device		*ongoing*		
3e. Determine loading conditions for in vivo studies	completed			
3f. Perform in vivo studies		*ongoing & planned*		
3g. Perform analysis of mechanics		*ongoing & planned*		
TASK 4 – Aim 3: Injury Studies for Temporal Characterization (Years 2-4)				
4a. Identify injury conditions			*ongoing & planned*	
4b. Perform tissue assays for Aim 2		*ongoing & planned*		
4c. Perform injuries			*ongoing & planned*	
4d. Perform tissue assays				*planned*

for Aim 3	
4e. Integrate findings from Aims 2 and 3	*ongoing & planned*
TASK 5 – Publish Findings from Aims 2 & 3 (by end of Year 4)	
5a. Identify potential publications	*ongoing & planned*
5b. Publish findings from Aim 2	*ongoing & planned*
5c. Submit findings from Aim 3	*ongoing & planned*
TASK 6 – Aim 4: Refine & Simplify Model System for Users (Years 2-4)	
6a. Initiate cost-analysis of device design	*ongoing & planned*
6b. Seek additional funding for prototyping if needed	*planned*
6c. Initiate analysis of proposed scaling algorithms	*ongoing & planned*
6d. Integrate injury risk evaluation analysis	*planned*
6e. Determine risk evaluation algorithms	*planned*
6f. Complete device development	*ongoing & planned*
6g. Distribute scaling laws	*planned*
6h. Complete software	*planned*
6i. Produce exposure guidelines	*planned*

Task 1

Work under **Task 1** corresponds to obtaining regulatory approval for both the animal studies (**Task 1a**) and for review of the human data from USAARL (**Task 1b**). In our prior summary we reported having obtained approval from both the University of Pennsylvania and USAMRMC for the rat studies. Accordingly, **Task 1a** was previously **completed**.

Work under **Task 1b** includes obtaining regulatory approval for use of the human data from USAARL and is ***ongoing***. During the last year, our collaborators at USAARL (Dr. Chancey et al.) have been actively working to obtain such approval but it has been delayed. An MTA between the University of Pennsylvania and USAARL was fully executed in January 2012 to loan Penn the de-identified data (e.g., accelerometer, 3-D position, EMG, force, ECG, internal pressure vehicle acceleration profiles) collected under DAMD17-91-C-1115 'Development of a Standard for the Health Hazard Assessment of Mechanical and Repeated Impact in Army Vehicles" (see Appendix A1 for copy of fully-executed MTA). With that approval in hand, we obtained datasets from USAARL in April 2012. Since that time we have been working on analyzing those data and summarize that work under Task 2 below.

Task 2

Work under **Task 2** corresponds to Aim 1 which includes several sub-tasks of reviewing data related to symptoms (**Task 2a**) and analyzing existing data acquired previously at USAARL (**Task 2b**). Work under **Tasks 2c and 2d** includes running new simulations on the MARS at USAARL, based on the findings from Tasks 2a and 2b. Since approval was delayed for review of the human data, work on Tasks 2a and 2b are still ongoing and the remaining Tasks in Aim 1 are also delayed. Accordingly, in the last project period (since April 2012) we have focused most activities under Task 2b. Accordingly,

we summarize those ***ongoing*** activities here. As described in detail in our previous report, we have been working with datasets from USAARL previously collected by the British Columbia Research Inc. (BCRI). All data are de-indentified and are from the BCRI research protocol on repeated mechanical shock. The data we have been working from are from a series of studies that applied a range of shocks (amplitude and frequency) to subjects using the simulator. Subjects were exposed to a series of mechanical shocks in the x-, y-, and z-axes superimposed on a background of random vibration. Data includes experimental and calibration data from one short duration (ST) and two long duration (LT) experiments: **ST1**, **LT3**, and **LT4**. We have focused primarily on the ST1 studies, which involved three 35-minute sessions per subject for 10 subjects. ST1 was focused on determining the relative response to a range of shocks from 0.5 to 4 G and 2 to 20 Hz, in order to determine the frequency and direction of shock most likely to be a health hazard. Also, that dataset can enable the evaluation of whether the relationship between shock amplitude and spinal response is linear or nonlinear. Briefly, the **experimental conditions** included individual shocks of amplitudes 0.5 to 4 G and the fundamental waveform frequency of 2 to 20 Hz was applied to the subjects in a single axis for each day of testing. Each type of shock was applied twice. A 1.5-minute swept sinusoidal 0.4 G signal from 2 to 40 Hz was applied in each positive axis direction. Shock signals were 5.5-minutes in duration and included 0.5, 1, 2, 3, or 4 G shock magnitudes at 2, 4, 5, 6, 8, 11, 15, or 20 Hz for a single axis and direction.

Since April, we have worked collaboratively with personnel at USAARL (Chancey, Dorman, Shivers) to understand, evaluate and synthesize these data. In addition, in August 2012, Dr. Shivers and Mr. Dorman came to Penn for 2 days to work on data analysis and interpretation of these datasets and those we are generating in Tasks 3 and 4. A variety of types of data was acquired using an array of different **instrumentation** approaches. ECG, EMG, force, acceleration, and internal pressure data were collected at 500 Hz. In addition, for the ST1 tests only, positional data were collected using the Optotrak at 200 Hz. Acceleration was measured at the seat and at the thoracic and lumbar spines. The calibration data includes acceleration during a brief pull and release of the skin next to the accelerometer in order to characterize the skin-accelerometer system. Optotrak position data were measured during ST1 using markers on the spinous processes at C7, T4, T6, T8, T9, T10, T12, L1, L5, and on the seat. We have focused our initial efforts on the accelerometer and positional data. In particular, we have analyzed transmissibility at T3 and L4, corresponding to where the accelerometers were placed, and using the Optotrak data at T4 and L5, have made comparisons of these two transducer and video approaches to making the same measurements. This is particularly relevant as it corresponds to our activities in the rat model (under Task 3).

In summary, of the 10 subjects, only 9 were found to have accelerometer data so we report findings based on that sample size (n=9). Acceleration along the z-axis was acquired at 500 Hz and each file contained 35,000 samples, corresponding to 30 seconds of data. During that time, a sine sweep from 2 to 20 Hz was applied to the seat. Analysis was performed according to the broad protocol description below:

1. 'Bin' every 1000 samples at each level for each of the seat and spine accelerometer,
2. Take RMS acceleration of the corresponding seat and spine accelerometers,
3. Divide RMS Spine/RMS Seat to obtain transmissibility,
4. With those 1000 samples use FFT to determine dominant frequency,
5. Plot against transmissibility for each frequency.

For the most part, the individuals demonstrated consistent responses (Figures 1 & 2). However, subject #10 exhibited greater T3 transmissibility at 4 Hz than other subjects and both subject #10 and #4 exhibited different responses at L4 than the rest of the cohort (see Appendix A2 for summary of individual transmissibility responses).

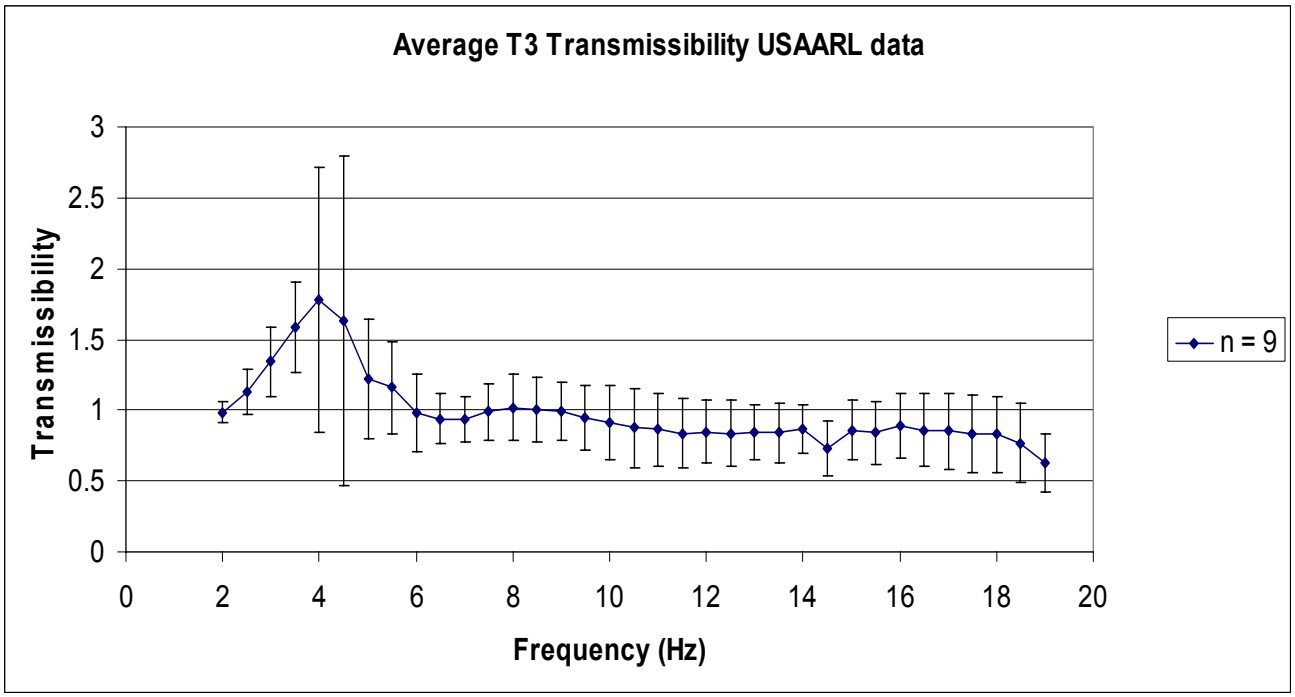

Figure 1. Average transmissibility response at T3 for a seat acceleration along the z-axis of 9 human subjects.

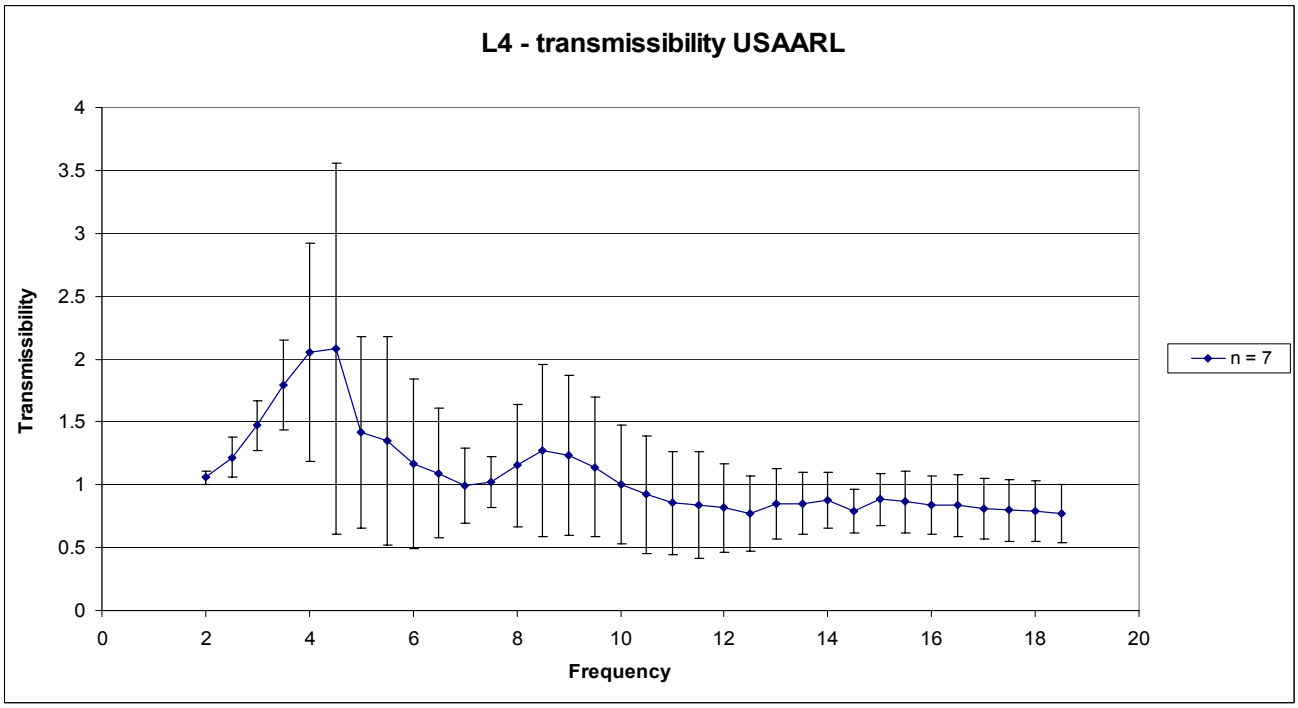

Figure 2. Average transmissibility response at L4 for a seat acceleration along the z-axis of 9 human subjects.

In addition, we find that the accelerations and transmissibility responses determined using imaging and accelerometer data are in very close agreement at both the thoracic and lumbar levels (Figures 3 & 4). Of note, the image-based responses using the Optotrak result in a slightly higher transmissibility value at each frequency but the trends are nearly identical and the degree of variation is

the same for both methods of analysis. This is quite encouraging in terms of enabling future interpretation and integration with other studies using only one or the other method of tracking whole body mechanical responses to vibration.

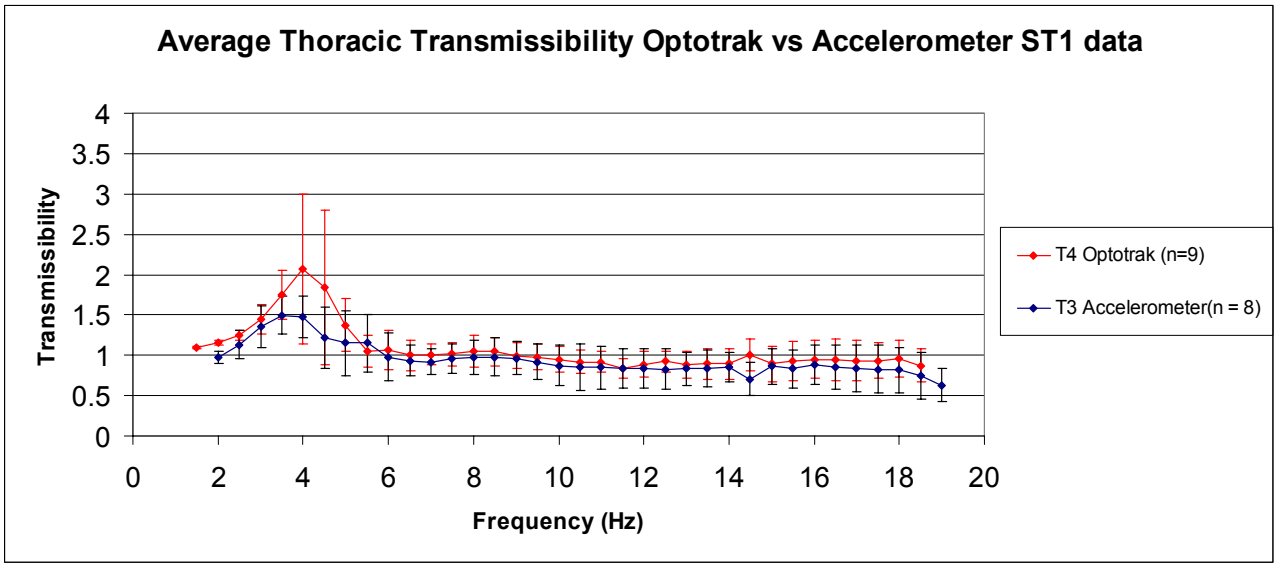

Figure 3. Average thoracic transmissibility responses using video (Optotrak) and accelerometer-based methods show very good agreement, with the Optotrak-derived response being higher at the apparent resonant frequency.

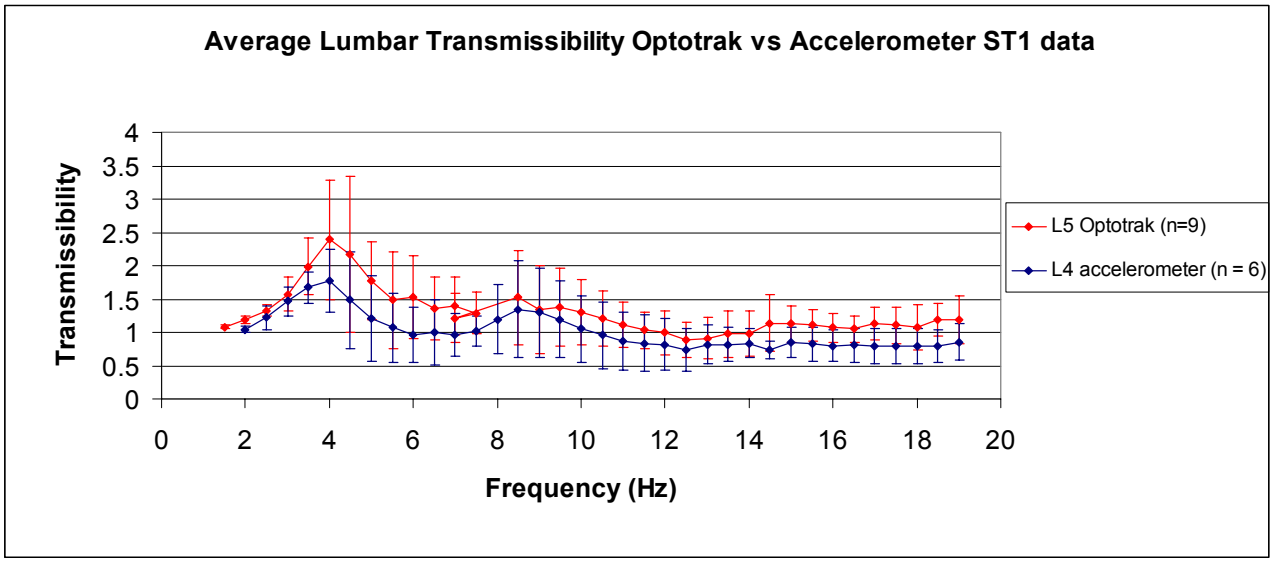

Figure 4. Average lumbar transmissibility responses using video (Optotrak) and accelerometer-based methods show generally good agreement, with the Optotrak-derived response being higher at the apparent resonant frequency.

We continue to work with USAARL to better understand and analyze these datasets. In particular, we continue with activities under **Task 2a** and **Task 2b** to incorporate the EMG data in order to better define the spinal response to these exposures. In addition, we are further verifying and analyzing these findings we have to date that appear to indicate the human resonance frequency to be at 4 Hz (Figures 1-4). Those efforts are still ***ongoing*** and work in the rest of Task 2 remains in the status of ***planned***. We are currently planning a visit to USAARL in order to better position ourselves for the activities that are upcoming in **Task 2c** and **Task 2d**. In addition, we are preparing an abstract on this work that will be submitted in December 2012.

Task 3

Work under **Task 3** corresponds to Aim 2 of the proposal and focuses on refining our previously developed experimental methods to impose controlled vibration in vivo and to evaluate pain and functional outcomes for vibration and jolt loading to the neck and low back. With **Task 3a and Task 3c** completed in the prior report, and the initial device and exposure conditions established for imposing vibration, efforts in this task in the last year focused primarily on developing and refining two specific exposure protocols and defining the related biomechanics and behavioral responses associated with them (**Tasks 3e-3g**). For brevity we do not re-describe our system since it was described in great detail in last year's report and can be found in our publications [1-4]. Further, based on our prior work [1-3], we elected to move forward using a 15 Hz vibration.

Based on our prior pilot studies, we performed studies using two different vibration exposures applied under inhalation anesthesia (4% isoflurane for induction, 3.5% for maintenance). Separate groups of rats were exposed to either a repeated daily whole body vibration (n=6) or an intermittent vibration applied only on 2 days with a rest-period between them (n=8). For each vibration exposure, the rat was vibrated at 15 Hz with a magnitude of 1.5 mm (corresponding to an acceleration of 0.63±0.09g) for 30 minutes. For the repeated exposure model, vibration was applied daily for 7 consecutive days; the intermittent exposure model used an exposure on day 0 and again after a 6-day rest after the first exposure. A sham control group (n=4) underwent anesthesia treatment matching the same timing of the repeated vibration group. During each exposure, the rat was placed in a prone position and secured to a customized acrylic platform by velcro straps. The platform was rigidly fixed to a linear servomotor (MX80L; Parker Hannefin) that was programmed and controlled by a digital driver (VIX500IH; Parker Hannefin). A laser LVDT (LTC–050-10; MTI) also tracked the platform motion. Two miniature quartz shear accelerometers (ACC104A; Omega) quantified accelerations of the plate and the rat; one accelerometer was affixed to the plate and the other was embedded in a velcro strap secured to the rat at its lumbar region. Markers were placed on the base plate and the lumbar accelerometer and were tracked by a high speed CCD camera (VRI-MIROEX1-1024MM; Phantom; 640X480) during vibration.

Behavioral sensitivity was assessed by measuring mechanical hyperalgesia in the forepaws and hindpaws on all days. Prior to vibration exposure, rats were also assessed for hyperalgesia to provide a baseline measurement to serve as an unexposed control response for each rat. Methods to measure hyperalgesia were adopted from Chaplan's up/down method and have been previously reported and validated [5,6]. The response threshold was measured using increasing strengths of von Frey filaments, ranging from 0.6 to 26 g, to stimulate the plantar surface of the paw. The lowest-strength filament to provoke a positive withdrawal response was taken as the response threshold if a withdrawal response was validated by application of the next higher filament. Each testing session consisted of three rounds of five stimulations to each forepaw, with at least a 10-minute rest period separating each round. The forepaw and hind paw responses for each rat were averaged by group on each testing day.

Sustained hypersensitivity is induced in both the hindpaw and forepaw (Figures 5 & 6). Vibration exposure induces sensitivity in the hindpaw as early as day 1 in both the repeated and intermittent groups. However, only the repeated exposure is significantly different from sham responses over all days ($p=0.039$) (Figure 5). Although hyperalgesia is immediate (day 1) after repeated exposure and the reduction in withdrawal threshold is sustained through the entire testing period until day 14, the threshold responses induced by sham remain at baseline levels for all postoperative days. Intermittent exposure induces sensitivity that is transient after a single exposure with a significant reduction only being sustained through day 5 ($p=0.004$). Interestingly, a second vibration exposure induces longer lasting sensitivity sustained through day 14 compared to the first exposure ($p=0.039$), but no additional increase in sensitivity beyond that observed after the first exposure (Figure 5).

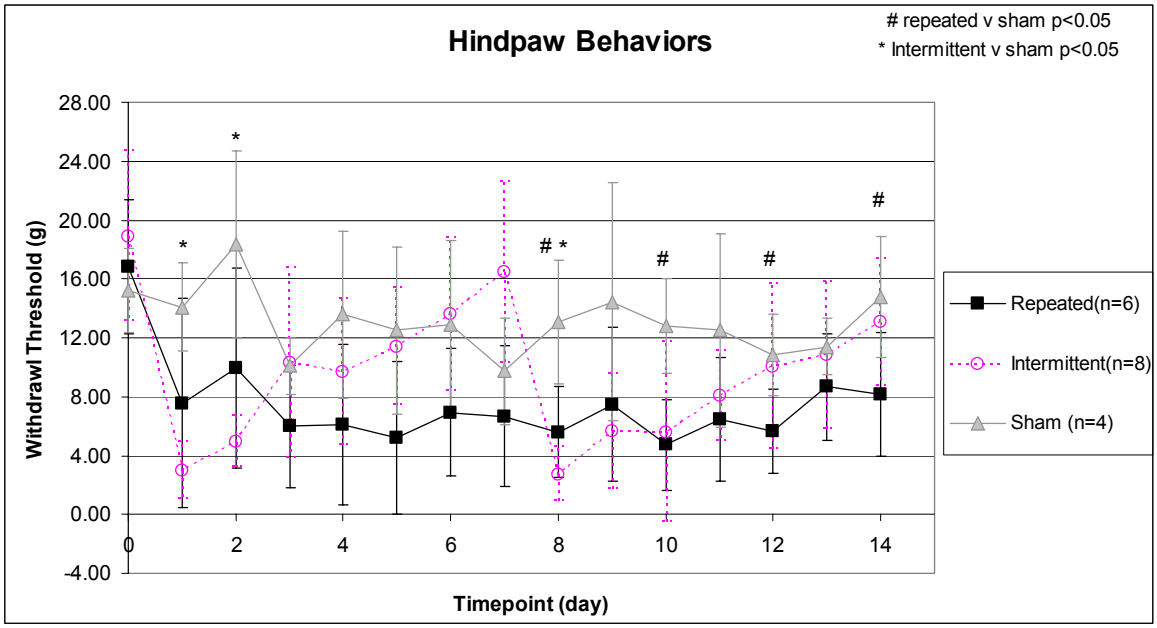

Figure 5. Behavioral sensitivity in the hindpaw following repeated daily vibration exposure, an intermittent exposure on days 0 and 6, and sham control.

Overall, both repeated ($p<0.0001$) and intermittent exposures ($p=0.043$) induce increased hypersensitivity in the forepaw compared to sham and are different from each other ($p=0.026$) (Figure 6). Repeated exposure responses are significantly different compared to sham on all days except day 4, whereas intermittent exposure is only different on days 1, 2, 3, 8-11 (Figure 6). Similar to the hindpaw, repeated exposure reduces the forepaw withdrawal threshold below baseline levels through the entire testing period regardless of loading or rest. Intermittent exposure induces only transient sensitivity in the forepaw. The rate of recovery back to baseline is significantly ($p=0.036$) slower after the second exposure than the first.

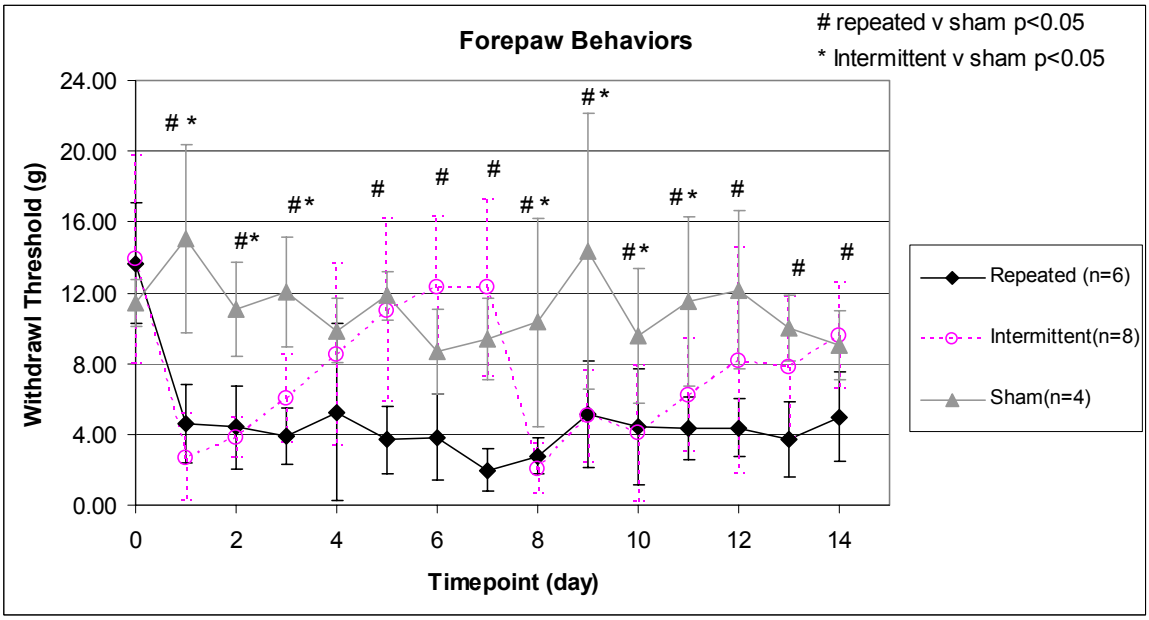

Figure 6. Behavioral sensitivity in the hindpaw following repeated daily vibration exposure, an intermittent exposure on days 0 and 6, and sham control.

For all vibrations, 30 seconds of accelerometer data and 12 seconds of video data were analyzed. Marker displacements of the plate and lumbar accelerometer were determined from the images by digitizing their positions relative to stationary reference markers in each image frame using ProAnalyst software. Both image and accelerometer acceleration data were filtered using a 5th order Butterworth bandwidth filter. The root mean square (RMS) acceleration was calculated for each exposure session and averaged for all days of exposure. Peak-to-peak displacements were determined using the LVDT and video marker data. There was no difference in any of the mechanical injury metrics for an exposure session within an injury group. The mean RMS lumbar acceleration magnitude for the repeated exposure (6.18±0.69 m/s^2) was not different from the acceleration magnitude for the intermittent exposure (6.16±1.01 m/s^2). Similarly, the mean horizontal displacement for the repeated exposure (1.93±0.46 mm) was not different from the displacement for the intermittent exposure (1.44±0.22 mm). An example of raw acceleration data and summary information for these studies can by found in Appendix A3.

Similarly, using the video data we investigated relative kinematics in the cervical and lumbar spines, in order to assess whether the behavioral differences that were observed in the forepaw and hind paw responses are attributable to differences in the biomechanical loading of the spines in those regions. We analyzed the resting and maximal and minimal lengths of the neck and note that both spinal regions undergo significant ($p<0.02$) changes in length during compressive cycles, and that the cervical spinal region also undergoes significant extension ($p=0.01$) during the tensile cycles (see Appendix A3 for data). We continue to integrate these biomechanical and behavioral data in order to better understand the biomechanical mechanisms that may explain the pain onset and maintenance in these injury exposures.

With the biomechanical findings from studies described above and in our previous report, we have continued to carry out studies to help define the rat response in the context of the human response, as well as to exploit those methods which are possible to do in the rat that are not manageable or pragmatic in the human. These efforts under **Tasks 3f and 3g** are ***ongoing***. One such activity has been to attach an accelerometer directly to the rat spine in order to evaluate how well the accelerometer affixed to the skin is approximating the spine's response (Figure 7). We have performed such studies to define the transmissibility response of expired rats (n=4) using both approaches. Interestingly, it seems that the transmissibility response defined by the skin's accelerometer is actually lower than that for the spine (Figure 8). Nonetheless, the resonance frequency is the same for both cases and the difference at 15 Hz is negligible (Figure 8). We are continuing these studies and will be expanding them to understand and define the cervical spine responses.

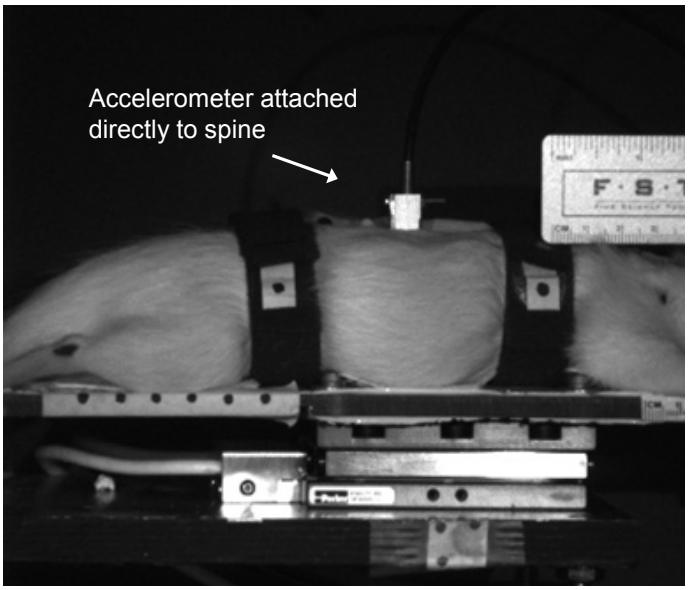

Figure 7. Image showing rat mounted on vibration device with motion tracking markers and accelerometer mounted directly to the spine.

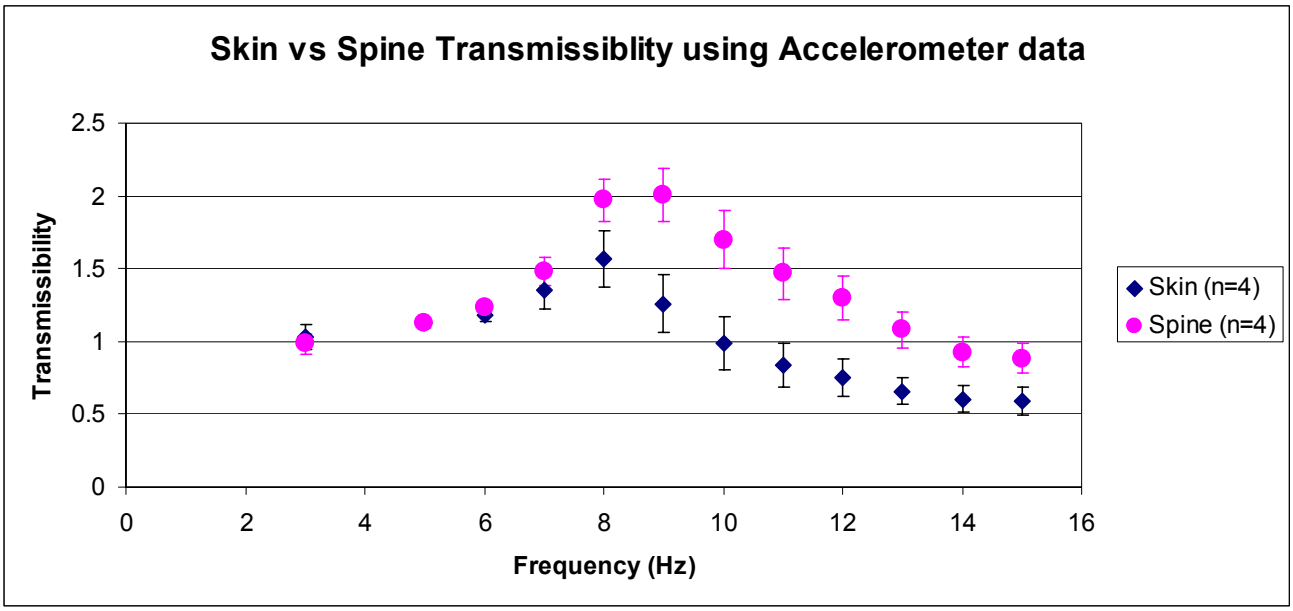

Figure 8. Transmissibility of the rat using accelerometer affixed to the skin and spine in matching studies using expired rats.

Task 3b focuses on establishing scaling criteria between the rat and human. As previously described our work has focused on two areas; (1) defining the anatomy and geometry of the rat spine in order to compare the size, shape and relationship of anatomical features to the relevant anatomical features of the human and (2) developing a mathematical model of the rat spine for vibration along the long-axis of the spine in order to investigate aspects of this model system for easy comparison to the human. In the last year, work has been undertaken in both of these areas and the anatomic scaling work is just **completed**, while the mechanical modeling work is ***ongoing*** as we integrate these findings with the mechanical studies in **Task 3g**.

The cervical and lumbar columns of 5 male Holtzman rats (328±19g) were harvested and their paraspinal muscles removed. The exposed osteoligamentous spinal columns were scanned using a high-resolution microtomographic system (vivaCT 40; Scanco) in multi-slice mode. A lateral scout view was taken to identify and capture the C3-C7 cervical and L1-L5 lumbar levels. DICOM images were acquired at a slice thickness of 0.38 μm and a 1024x1024 axial field of view, with 32-bit-gray levels to enable segmentation of the bony structures using the ITK-SNAP software. A semi-automatic segmentation process based on the gray-level intensity of the μCT images enabled the identification, delineation, and reconstruction of the individual vertebra at each spinal level. The 3D reconstructed vertebrae and spinal columns were imported into the 3-matic software (Materialise; Leuven, Belgium) for quantitative measurements of their bony anatomy. Several measurements were performed in both the axial and sagittal planes to describe the dimensions of the vertebrae and intervertebral discs [7].

Using the axial view, the vertebral body, spinal canal, and vertebra depths were each defined as the corresponding maximum lengths along the antero-posterior direction at the midsagittal plane (midline). Similarly, the widths of the vertebral body, spinal canal, and vertebra were also quantified using the maximum lateral dimensions measured normal to the midline. Interfacet distance was defined as the maximum distance between articular masses measured normal to the midline. The pedicle angle was defined as the angle between the midline of the pedicle and the vertebral body. All width measurements were normalized by vertebra depth at each level to account for differences in measurements due to animal variability. Measurements of the vertebral body and intervertebral disc heights were also made in the sagittal plane. Vertebral body height was measured at the anterior edge of each vertebra as the distance between the superior aspect of its upper endplate to the inferior aspect of its lower endplate. Intervertebral disc height was measured at the anterior edge of adjacent vertebrae as the

distance between the aspects of the upper vertebra's inferior endplate and the lower vertebra's superior endplate. All height measurements were also normalized by the corresponding vertebral body depth to permit comparisons with human spine anatomy. The initial work with this approach applied to the cervical spine was presented as a poster in 2012 [7] and we have expanded the work to include lumbar assessments. A summary of findings to date is provided in Appendix A4. We are currently comparing these rat-derived normalized measurements to similar such measurements in the literature for the human spine. That analysis will be completed in the next month and we expect to submit a manuscript by the end of the calendar year. From our direct comparison previously to the cervical spine in humans [7], we are encouraged that a direct scaling algorithm will be easily derived since the rat spine is closer to the human than previously suspected.

Task 3e is generally **completed** now that we have determined 15 Hz and have established our repeated and intermittent exposures. The work with the intermittent exposure has been the first step in implementing the studies for a single jolt and so we have studies planned for the next year to investigate the effect of a single jolt of greater based on our finding of transient sensitivity for the 15 Hz 30 minute exposure (Figures 5 & 6). These studies are ***ongoing and planned*** in order to determine the full set of loading conditions for the in vivo studies to be completed later in **Task 3f**.

In addition to the analysis of the kinematics and kinetics already described above for the vibration studies in vivo and the transmissibility studies under **Task 3b**, we have continued the studies we initiated in Year 1 to develop lumped mass models simulating our vibration system. Three mechanical-analog models of the vibrated rat were developed as mass-springs-dampers systems. Using the dynamics equations, the theoretical expressions of the transmissibility and phase shift were expressed and compared to their experimental counterparts. The experimental transmissibility and phase shift were based on the analysis of the filtered accelerations of the plate and the rat. Since the rat was equipped with only one accelerometer, we limited our models to be 1-DOF models. The three models are depicted in Appendix A5.

Having established the theoretical models, we performed comparisons with each model against the experimental data from one rat (#T2) (Figures 9-11); we are in the process of refining these models based on those runs and so the responses indicate outcomes but the models have not been optimized. Nonetheless, each of these models are performing well given the fact that this is a 1-DOF system and our own biomechanical data support at least a 2-DOF system.

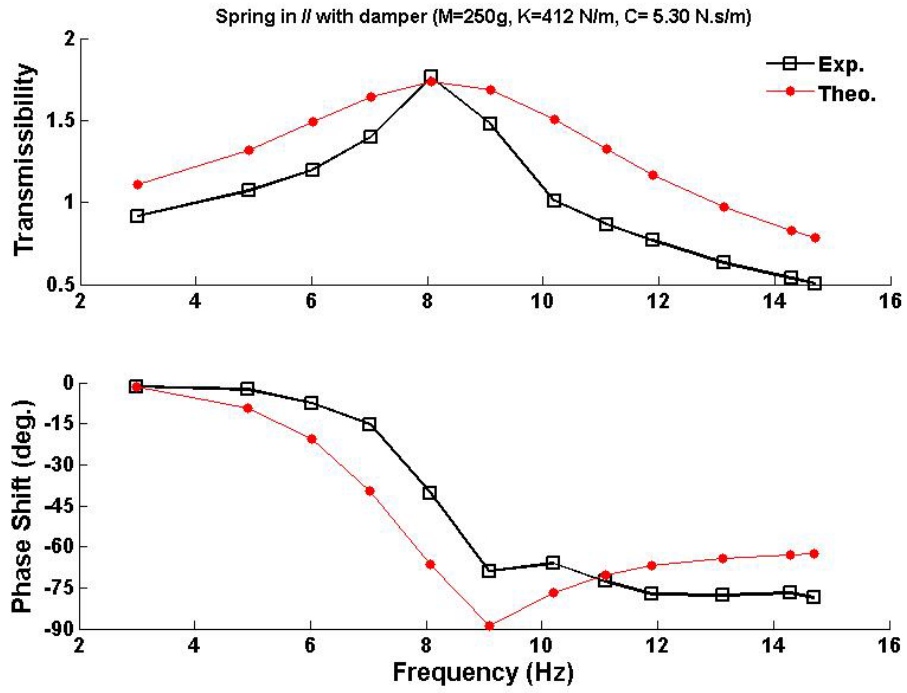

Figure 9. Response using Model #1, with the spring in parallel with the damper. There is general agreement in both the transmissibility and phase shift.

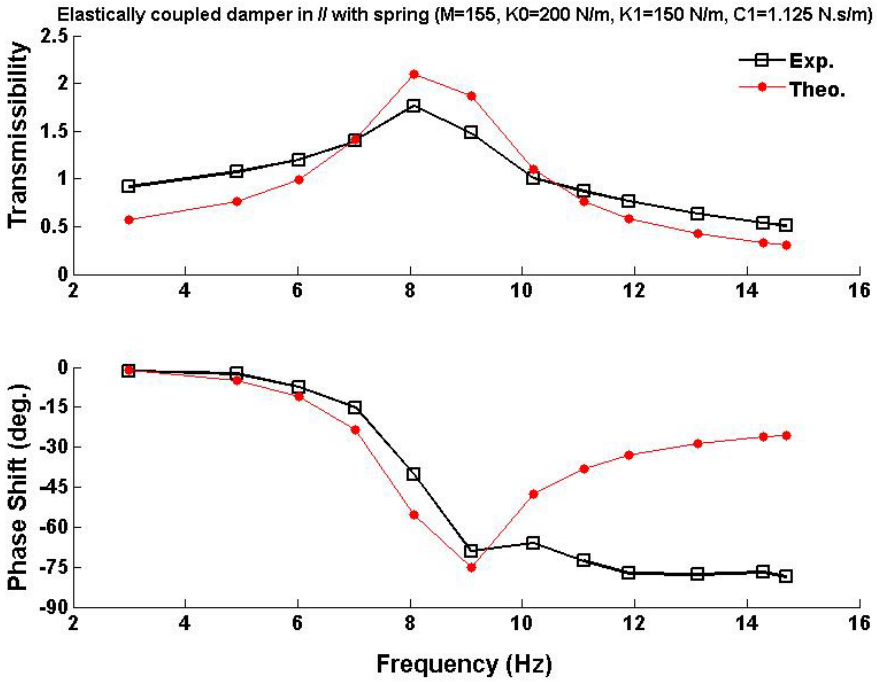

Figure 10. Response using Model #2 showing very good agreement in matching the transmissibility response. However, the phase shift is not matched at higher frequencies.

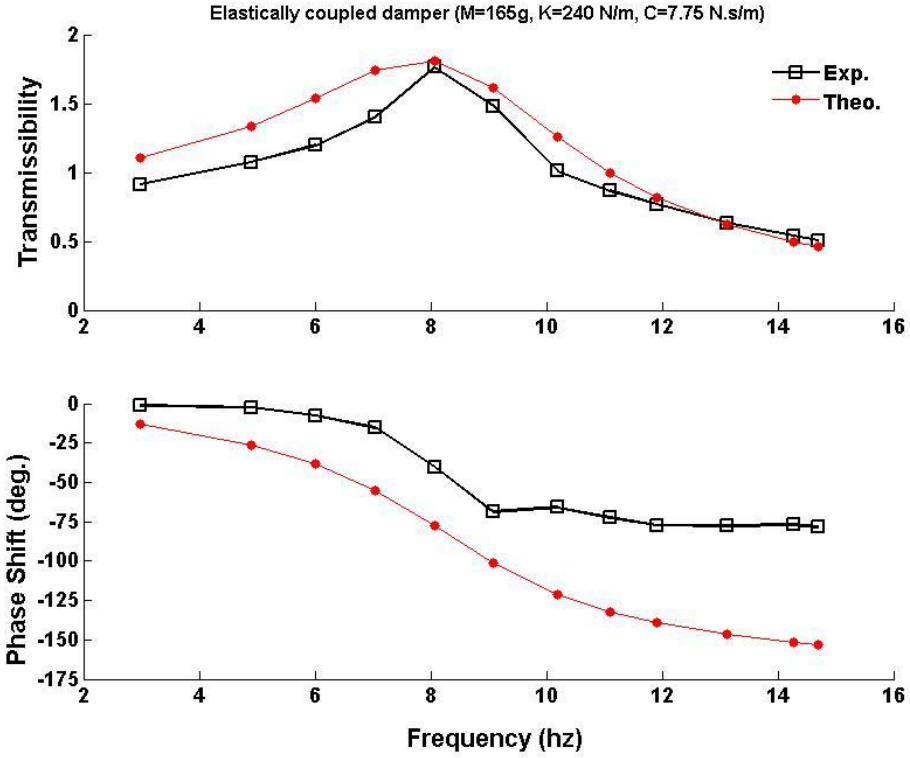

Figure 11. Response using Model #3 showing very good agreement in matching the transmissibility response. However, the phase shift is not well-matched.

Based on these earlier results, the experimental transmissibility and phase shift response from other rats will also be analyzed to establish a corridor of the experimental responses. Then, the optimal values of the mass, spring, and damper coefficients will be calculated by minimizing the error between

the data points for both responses using least-squares methods. Activities under **Task 3g** are ***ongoing*** and will be completed according to the original time line by the end of Year 3.

Task 4

Work under **Task 4** corresponds to Aim 3 which involves the temporal characterization of responses in relevant tissues in the periphery (appendages), spine, and central nervous system following the vibration exposure. In the past year we began to generate those tissues for relevant time points for the whole body vibration exposures (daily and intermittent exposure models). Of note, **Task 4a** is ongoing and planned with regards to the specific jolt injury condition, but with the injury conditions of the whole body vibration already defined under **Task 3**, we are active in generating tissue for those injury conditions under **Tasks 4b-4c**. Work with the jolt exposures under **Tasks 4a-4e** are currently planned for initiation in the next year.

At each time point of tissue harvest, we collect a variety of tissues, including the brain, cervical and lumbar spinal cord enlargement, cervical and lumbar discs, paraspinal muscles in both regions of the spine and the gastrocnemius muscle since it is close to the region where behavioral sensitivity is measured in Task 3. Also, when available, we also harvested DRG samples but due to their small size it is not always possible. We have focused on collecting tissue at several time points throughout the exposure, based on the behavioral outcomes observed for pain onset and/or resolution: day 1, day 7, day 8 and day 14. We also include a tissue from sham anesthesia groups at each time point. A complete summary of animal numbers (having the above listed tissues harvested) for each group to date is provided in Appendix A6. In addition, we have scheduled another series of studies that will generate tissue from 4 rats at day 8 in the repeated 15Hz daily exposure group and sham group, as well as 2 rats each in the intermittent exposure group at day 7 and day 8. These are currently underway and will be completed on November 21, 2012.

Initial studies probed spinal cord and spinal discs at 7 days after the cessation of the repeated daily exposure. Since these studies have been previously presented at scientific conferences [1,4], we present in detail here the findings since that time for which we do not have published materials. However, briefly, those publications document significant modifications of COX2 in the spinal cord and the neurotrophic factors, BDNF and NGF, in the spinal discs in the cases of chronic pain, suggesting that inflammation and nerve outgrowth may be induced in this painful model. Based on those findings we have initiated parallel studies of spinal cord and intervertebral discs.

The spinal cord tissue (n=7) harvested from rats that were exposed to a whole body vibration at 15Hz for 30 minutes daily for 7 days was compared to an additional group of rats (n=6) that were only exposed to anesthesia for that same period of 7 days to serve as the sham control group. Both lumbar and cervical spinal cord samples were assayed to quantify BiP, a marker of activation of the cellular stress response, using western blot analysis. BiP expression for each sample was normalized by β-tublin levels and compared between groups using t-tests. BiP expression levels in the lumbar spinal cord were significantly lower ($p=0.012$) in the vibration group (0.008 ± 0.004) than in the sham control group (0.028 ± 0.016), but unchanged in the cervical region (Figure 12). BiP has been shown to be modulated in association with other painful injuries. In fact, a painful facet joint injury upregulates BiP expression in neurons of the dorsal root ganglia. However, the current finding of decreased BiP in the spinal cord may suggest that cells in the spinal cord may be damaged by the vibration exposure, leading to the decrease in BiP. Additional studies are needed to define the time course of development of this change, as well as studies to define in which spinal cells these changes are occurring. Regardless, this novel finding was included in an abstract that we recently submitted for an upcoming scientific meeting.

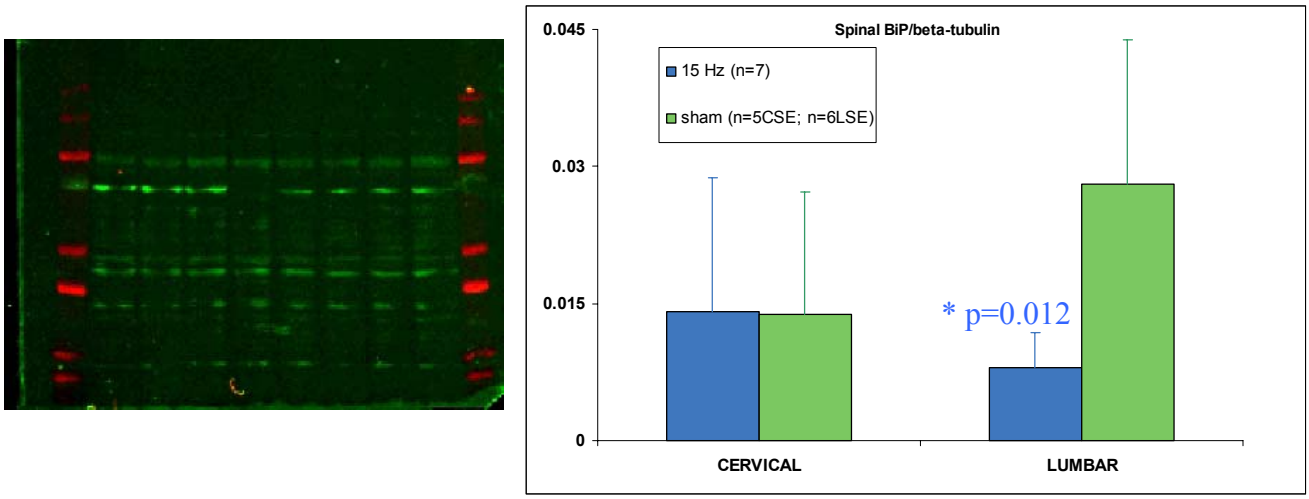

Figure 12. Western blot (left) and quantification of protein levels (right) of BiP in the cervical and lumbar spinal cord of sham and 15Hz vibration exposures, showing no change in the cervical spinal cord, but a significant (p=0.012) decrease in spinal BiP following a 15Hz vibration exposure protocol that induced sustained pain.

Our initial observations of modifications in neurotrophic factors in the intervertebral discs of vibrated rats in association with the presence of pain [4] suggest that whole body vibration may modulate the structure, function and physiology of the intervertebral discs in our model. Those earlier studies were based on findings using western blot assays and were not able to investigate the structure of individual discs and any regional variation, change in height, and/or nerve ingrowth. Accordingly, in the last year, we have focused efforts on developing protocols to fix full spinal columns to undergo histology and immunostaining.

Because of the behavioral findings (Figures 5 & 6) indicating cervical responses may be more robust than lumbar, we focused initial studies on cervical spinal columns. Full spinal columns from both vibrated and sham control rats were harvested from rats on day 7 after either a daily 15Hz vibration or anesthesia exposure for 7 days. Spines were harvested via fixed perfusion with 4% paraformaldehylde and columns were dehydrated in 30% sucrose for 7 days before being placed into a decalcifying solution, 10% EDTA, for three weeks. After decalcification, columns were placed again in 30% sucrose before being sectioned in the sagittal plane at a 20µm thickness, starting in the midline.

A hematoxylin and eosin staining protocol was used to help expose and enhance visualization of structural changes in the intervertebral discs, particularly the nucleus pulposus and the annulus fibrosus. We have developed methods to process full cervical spines keeping intact several spinal levels (Figure 13). Additional images of motion segments and discs can be found in Appendix A7. Further, with these methods in place, we have begun to develop methods exploiting the structural features visualized by these methods to make measurements of disc height and inflammatory cell infiltration to serve as proxies to evaluate any changes in the structure and function of the discs. These activities are ongoing and we expect to make substantial progress over the next several months.

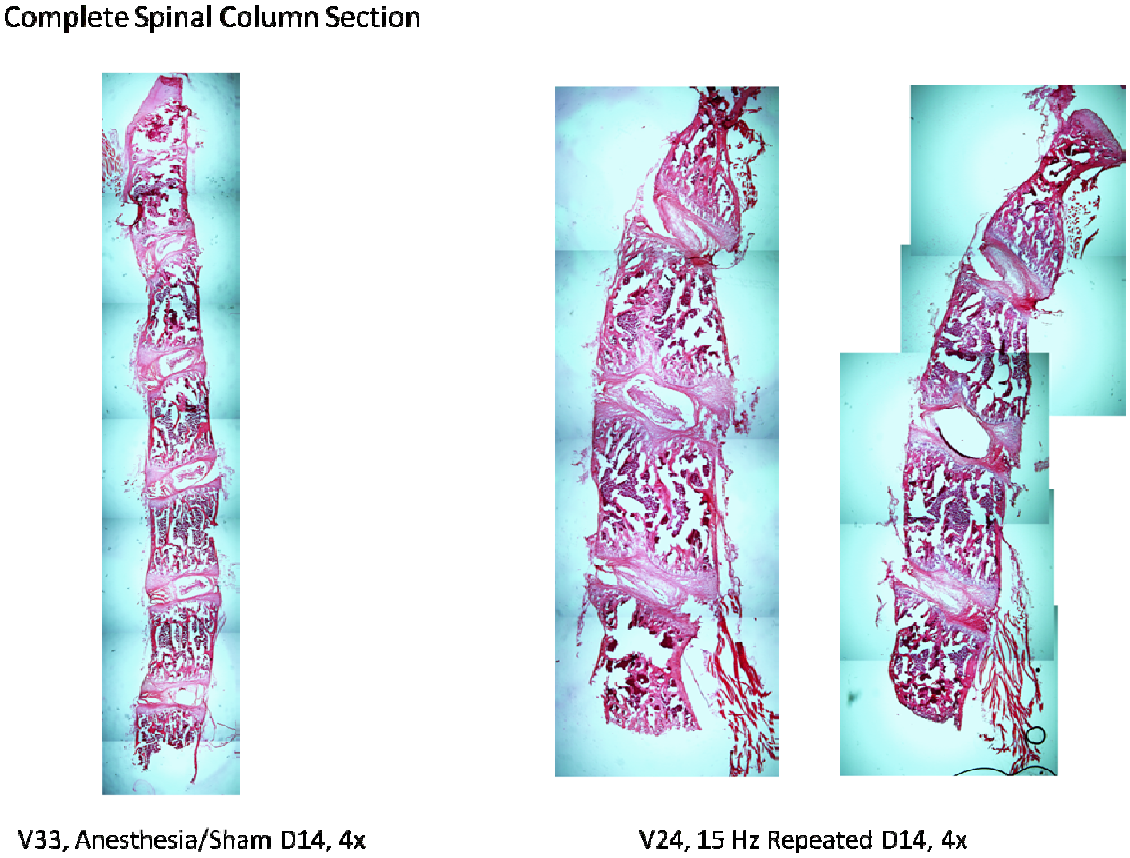

Figure 13. Structural staining of cervical spinal columns from rats harvested on day 14 after 7 days of anesthesia (V33) and 15Hz vibration (V24). Using these stains, cytoplasm is red and chromatin is blue.

Following the western blot analysis of disc sections for the proteins, BDNF and NGF, that we previously reported in our prior progress report and in our abstract [4], we have been developing protocols for immunofluorescent detection of these same proteins, and other markers of nerve fibers, on the disc sections in order to be able to localize these proteins in the disc. In addition to staining for proteins BDNF and NGF, sections are also labelled using β-III Tubulin (an axonal marker) and a marker previously used in disc innervation studies, GAP-43, which is taken as an indicator of nerve fiber outgrowth. Efforts are ongoing to optimize antibody dilutions, incorporate methods of antigen retrieval and develop methods of specific and sensitive staining. Currently, we are working with the following antibodies for fluorescent staining :

Mouse Anti-Mono- β-III Tubulin (Covance)
Rabbit Anti-BDNF (Abcam)
Rabbit Anti-NGF (Millipore)
Mouse Anti-GAP43 (Abcam)
Alexa-Fluor 488 goat anti-rabbit (Invitrogen)
Alexa Fluor 546 goat anti-mouse (Invitrogen)

In this report, we provide several of the images taken from the discs of cervical and lumbar spines from both vibrated and sham conditions as a summary of our current and ongoing progress (see Appendix A7). We have recently revised our immunofluorescent protocol to include an antigen retrieval step before labeling with BDNF, to promote cleaner staining (Figures 14 & 15). Lastly, in addition to

these approaches, we are also optimizing protocols to perform labeling using non-fluorescent methods, ie. ABC/DAB staining. These studies are ongoing and will continue in the next project period.

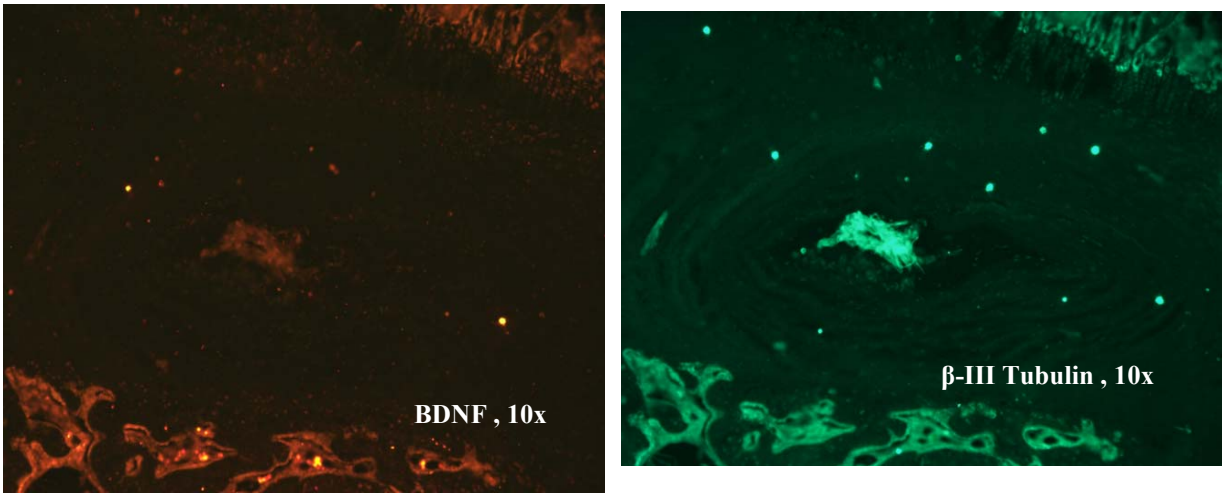

Figure 14. A lumbar disc from rat #V21 which had undergone procedures for an anesthesia/sham protocol. BDNF (1:500, Rhodamine) (**LEFT**) and Right, Beta-III-Tubulin (1:500, FITC) (**RIGHT**) labeling are shown, with 20 minute antigen retrieval, using Citrate Buffer at pH 6.

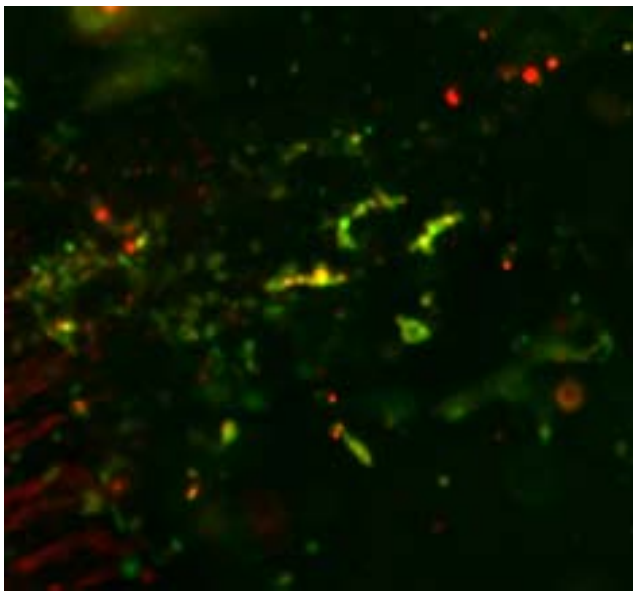

Figure 15. The same disc in Figure 14, at higher magnification, showing BDNF and Beta-III-Tubulin (1:500, FITC) merged (yellow), indicating some neurons positive for BDNF, but not all.

Both of these ongoing activities are direct extensions of work based on the findings we presented previously in abstracts (see Appendix A8). With the inclusion of these additional outcomes we are poised to prepare manuscripts summarizing the broader context and meaning of these modifications.

In the last year, we also initiated histological assays of the paraspinal and gastrocnemius muscles to develop methods to evaluate if and to what extent muscle injury is induced by these vibration exposures. We have been developing protocols for tissue harvest, sectioning and staining to examine muscle fibers based on the hypothesis that muscle atrophy is induced by repeated vibration. To this end, we have harvested the paraspinal muscles in the lumbar region and sectioned them perpendicular to the muscle's long-axis (Figure 16). From initial studies, we have determined that sections at 10-16 μm are

adequate and appropriate for initial screening of muscle fiber density. For example, using hematoxylin and eosin stains, it is apparent that there is not recruitment of inflammatory cells following the painful whole body vibration (Figure 16). Further, initial studies suggest that there may be increased spacing between muscle fiber following a whole body vibration exposure, compared to normal uninjured controls (Figure 16). We are currently carrying out additional studies to perform a more detailed investigation of fiber size, number, density and general muscle health in the groups from Task 3. In addition, we will initiate studies of the gastrocnemius muscles in the next year.

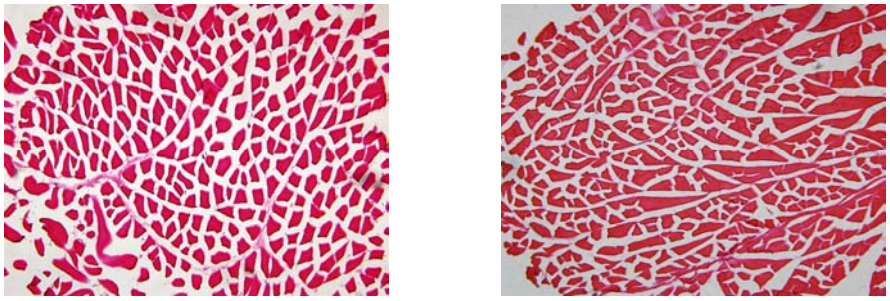

Figure 16. Images of lumbar paraspinal muscle following a 15 Hz repeated vibration and 7 day rest (LEFT) or normal tissue (RIGHT). There is more spacing between muscle fibers in the vibrated tissue than normal.

We have not yet probed the brain tissue but have developed protocols and such assays are planned for the next few months.

As mentioned above, additional studies at different relevant time points are ***ongoing*** and are ***planned*** for the remainder of the project period. However, based on the data already from the 15 Hz exposures in Task 3 and the findings summarized in our last report (and in the abstracts in Appendix A8), we are preparing a manuscript that includes the findings of neurite outgrowth in the disc. We expect to submit that paper in the next year and that several more will follow with the continued studies in Tasks 3 and 4.

Task 5

Work under **Task 5** corresponds to identification of publications for work from Aims 2 and 3 and is ongoing. It will be completed by the end of Years 3 and 4 as detailed in the original statement of work. To date, we have presented 6 abstracts, submitted 1 abstract, and are currently preparing 3 manuscripts, 2 of which will be submitted in the next 3 months. Please see Appendix A8 for the published abstracts. Please also see the Reportable Outcomes section for additional details.

Task 6

Work under **Task 6** corresponds to Aim 4 which broadly consists of efforts to provide the model system and software as resources for the broader scientific community. The majority of the specific sub-tasks of that Aim are largely planned for the remaining years of this project. However, given our early successes in developing a working system and identifying the conditions for use in Aims 2 and 3, we also continue with **Task 6a** and **Task 6c**. We continue these analyses and are investigating more economic options for components of our device. In fact, we have found a programmable shaker, with improved performance capabilities compared to the motor of our first-generation device. Further, that component is far more affordable ($3,865.50). We will continue these ***ongoing*** efforts over the next year. Work in **Task 6c** has been partially completed by our developing scaling relationships between the rat (from our μCT) and human (from literature) in Aim 2. Efforts under **Task 6c** are ongoing and will continue as originally projected to be completed before or by the end of Year 3.

All **other sub-tasks of Task 6** are planned for completion during or by the end of Year 4, according to the original timeline.

KEY RESEARCH ACCOMPLISHMENTS

- Established two different exposure profiles that impose sustained and transient pain, respectively, in the live rat.
- Determined the resonant frequency of the rat spine to be 8 Hz and for the human spine to be 4 Hz for vibration along the long-axis of the spine.
- Derived complete anatomic datasets quantifying the bony anatomy of the rat cervical and lumbar spines for direct comparison (and scaling) to the human spine.
- Developed 3 different lumped parameter mathematical models of the rat spine and initiated studies that indicate these simple models are fairly good at capturing the rat response.
- Determined several changes in tissue responses (spinal cord, disc and muscle) in association with sustained pain.
- Established methodology to enable assays of spinal disc materials.

REPORTABLE OUTCOMES

Manuscripts, Abstracts & Presentations

1. Guarino BB, Baig HA, Branconi JL, Winkelstein BA. Repeated Whole Body Vibration Exposure Induces Prolonged Mechanical Hyperalgesia & Increased Spinal COX-2: A Novel Rat Model. *Northeast Bioengineering Conference*, Philadelphia, PA, March 2012.
2. Gohkale AJ, Guarino BB, Winkelstein BA. The Rat as a Viable Model for Human Cervical Biomechanics: A Quantitative Anatomy Study. *Northeast Bioengineering Conference*, Philadelphia, PA, March 2012.
3. Baig HA, Guarino BB, Jaumard NV, Winkelstein BA. The Transmissibility Response of the Rat During Whole Body Vibration Along its Long-Axis. *Northeast Bioengineering Conference*, Philadelphia, PA, March 2012.
4. Freedman BR, Baig HA, Guarino BB, Winkelstein BA. Biomechanical Effects of Whole Body Vibration on Spinal Ligaments: A Potential Mechanism of Tissue Damage. *Northeast Bioengineering Conference*, Philadelphia, PA, March 2012.
5. Branconi JL, Guarino BB, Baig HA, Winkelstein BA. Painful Whole Body Vibration Increases NGF & BDNF in Cervical Intervertebral Discs in the Rat. *Northeast Bioengineering Conference*, Philadelphia, PA, March 2012.
6. Guarino BB, Baig HA, Jaumard NV, Branconi JL, Dorman DB, Shivers BL, Winkelstein BA. A New Model of Repeated Whole Body Vibration Exposure in the Rat: Biomechanical & Pain Responses. *Military Health System Research Symposium*, #12-023, Ft. Lauderdale, FL, August 2012.
7. Tanaka K, Baig HA, Guarino BB, Smith JR, Winkelstein BA, Jordan-Scuitto KL. Painful Whole Body Vibration is Associated with Decreased BiP Expression in the Lumbar Spinal Cord. *American Association of Endodontics Annual Session*, submitted.
8. Guarino BB, Baig HA, Jaumard NV, Winkelstein BA. Repeated daily exposure to whole body vibration induces sustained widespread behavioral sensitivity that is not induced by a single exposure. *To be submitted by December 2012.*

9. Baig HA, Guarino BB, Winkelstein BA. A transmissibility study of the rat spine: A potential relationship to pain.. *To be submitted by December 2012*.
10. Kartha S, Guarino BB, Baig HA, Winkelstein BA. Vibration along the spine induces permanent modifications of nerve growth factor and PAR2 in the disc that are associated with persistent pain. *To be submitted*.

Degrees Obtained Supported by this Award

1. Akhilesh Gohkale, MSE in Mechanical Engineering and Applied Mathematics, awarded 2012
2. Hassam Baig, currently an MSE student in Bioengineering, degree expected in 2013.
3. Kosuke Tanaka, currently an MS student in the Dental School, degree expected in 2013.
4. Nadia Garbhi, currently an MS student in the Dental School, degree expected in 2013.
5. Ben Freedman, PhD student did a research rotation working on this project in Fall 2011.
6. Lorre Atlan, PhD student did a research rotation working on this project in Fall 2011.
7. Sijia Zhang, PhD student doing a research rotation currently (Fall 2012) on this project.

Animal Model Generated

1. Protocol developed to induce sustained behavioral sensitivity following repeated daily vibration to the rat.
2. Protocol developed to induce transient behavioral sensitivity following a single vibration to the rat.

Research Opportunities Applied for or Received Supported by this Award

1. DURIP proposal submitted September 2012, for high rate tissue tester to ext4end activities under this award, application pending.

CONCLUSIONS

There is currently very little definitive mechanistic data defining the relationships between whole body or spine vibration, tissue responses (biomechanical and physiological) and pain. Considering that pain is a tremendous problem especially for the military personnel, we have developed a useful novel model platform to study how such exposures produce chronic pain. We **hypothesized** that a model of vibration and/or jolt induced pain could be produced in the rat that would simulate the human exposures. **Studies performed in the last year (in addition to those reported in our prior progress report) continue to support our hypothesis and have importance in moving the entire project forward**. Among the major findings of importance include the fact that even 30 minutes of vibration a day for only 7 days is sufficient to induce significant widespread behavioral sensitivity that is sustained for at least a week following the termination of vibration. A second major important finding is that a single vibration exposure also induces behavioral sensitivity that takes nearly a week to fully resolve. Further, when a second single exposure is presented, the time to recovery is longer than after the first exposure. These behavioral findings have the ***very important implication that repeated single (benign) exposures, even with an adequate rest period, do indeed have detrimental effects on the symptom development, progression and recovery***.

In addition, we have also found that a host of biochemical changes appear to be present in association with pain and are evident in the periphery and central nervous system. Interestingly, while the resonant frequency of the rat is at 8 Hz, the human spine resonates at ~4 Hz. This has important implications as we proceed with scaling our findings to the human. But it must also be noted that this difference may be due to the experimental set-ups of the two species with the rat in the prone position and the human seated. However, by our integrating human, rat and mathematical models together, we this project is posed to for the first time fully-define the consequences of vibration from a mechanical, functional and physiological perspective**.** In addition, these studies establish a strong and exciting foundation for the remaining in vivo and human studies which expand these studies to include additional exposures and to define the time course of physiological responses in the whole animal system.

Based on the activities during the last year, we do not have any modifications to the future work, only to recommend slight changes to the timing of activities for the future work. As indicated above, activities to obtain regulatory approval for the review of human data were delayed and so progress to date on Aim 1 (Task 2) is still ongoing. Therefore, efforts on Task 2 will continue in to Year 3 given this unforeseen delay in reviewing the USAARL data. We believe we are currently positioned to move that work forward in an effective and meaningful way, facilitated by the strength of our already heavily integrated collaborative teams. We continue our monthly conference calls to continue to discuss efforts in those studies and to prepare for the work and are planning a visit to USAARL. We continue to move all efforts forward as best as possible and will compensate for this delay by expended extra effort in other areas of this project.

Current risk assessment algorithms for pain and injury rely largely on speculative notions and standards for injuries that may not be relevant. Although vibration is a common experience while riding in vehicles, and standards have been developed to protect Soldiers from repeated jolts, they are not sufficient for current designs, nor do they address neck injury potential or the mechanisms by which tissue loading produces pain and/or injury. Also, there is no clear understanding of the physiological consequence of repeated sub-threshold loading to lowering the pain threshold. Accordingly**,** our in vivo model that mimics the biomechanical loading to the body enables studying how loading produces tissue injury, which tissues are injured, how pain develops, and which conditions place the military specialists at greatest risk for injury. The new knowledge gained from such an injury/pain model has direct utility for evaluating injury risks and developing potential therapeutics. Our findings to date already provide evidence that even low level vibration is sufficient to produce pain and that even a rest period that is long enough for symptoms to resolve is not sufficient to prevent the subsequent development of worse symptoms upon re-exposure. Our in vivo and mathematical models that have already been developed under this project have tremendous promise for providing major benefit to the military by identifying tissues at risk for injury and exposures which pose the greatest threats to producing pain.

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APPENDIX

A1. Fully-Executed MTA for Data Usage of USAARL Data by Penn.

DEPARTMENT OF THE ARMY
U.S. ARMY AEROMEDICAL RESEARCH LABORATORY
FORT RUCKER, AL 36362-0577

COVER SHEET
CRADA – MTA

[NOTE: This Cover Sheet is for internal management purposes only. It is not part of the Agreement and neither party is bound to anything contained in it]

Title: University of Pennsylvania (UPenn)

Effective Date: 11 January 2012 USAMRMC Control No. W81XWH-12-0091
Expiration Date: 10 January 2015 DA/TTPO Control No.

Primary NTIS Subject Code/Title: 95/Biomedical Tech & Human Factors Engineering
Secondary NTIS Subject Code/Title: 95O/General

Laboratory:
U.S. Army Aeromedical Research Laboratory
ATTN: MCMR-UAX-SI (Dr. Loraine P. St. Onge)
P.O. Box 620577
Fort Rucker, AL 36362-0577
Voice Phone: (334) 255-6906 FAX Phone: (334) 255-6983

Lab's Technical POC:
Dr. Loraine P. St. Onge
Science Information Center
U.S. Army Aeromedical Research Laboratory
P.O. Box 620577
Fort Rucker, AL 36362-0577Voice Phone: (334) 255-6906 FAX Phone: (334) 255-6983
E-mail: loraine.stonge@us.army.mil

Lab's Legal Counsel:
Commander, U.S. Army Medical Research and Materiel Command
ATTN: MCMR-JA (Mr. James Jurich)
Fort Detrick, MD 21701-5012
Voice Phone: (301) 619-9879 FAX Phone: (301) 619-5034

UPenn's POC:
Mr. W. Stuart Watson
Associate Director
Office of Research Services
University of Pennsylvania
Franklin Building Annex
3451 Walnut Street, Room P221
Philadelphia, PA 19104-6205
Voice Phone: (215) 573-6707 FAX Phone: (215) 898-9708
E-mail: wswatson@upenn.edu; epeloso@upenn.edu;
ba-towne@seas.upenn.edu; winkelst@seas.upenn.edu

Summary: The U.S. Army Aeromedical Research Laboratory is providing to University of Pennsylvania de-identified data collected under DAMD17-91-C-1115, "Development of a Standard for the Health Hazard Assessment of Mechanical and Repeated Impact in Army Vehicles," to be used to develop future methodologies and to define the most common and relevant loading profiles associated with low back and neck pain, in support of cooperative agreement W81XWH-10-2-0140, "Development of a Novel Translational Model of Vibration Injury to the Spine to Study Acute Injury In Vivo."

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT
FOR MATERIAL TRANSFER

This Agreement is entered into under the authority of the Technology Transfer Act of 1986, as amended. The parties of this Agreement are: University of Pennsylvania (Recipient) and the U.S. Army Aeromedical Research Laboratory (USAARL) (Provider).

With respect to Provider furnishing research Materials, the following data: de-identified data, including accelerometer and Optotrak three-dimensional position data measured at the seat and spine, EMG measured from back and abdominal muscles, force measured during EMG-force calibration, ECG, internal pressure measured with a rectal pressure probe, and vehicle acceleration profiles measured with accelerometers in ground vehicles, collected under DAMD17-91-C-1115, "Development of a Standard for the Health Hazard Assessment of Mechanical and Repeated Impact in Army Vehicles," (Data), and/or information relating to them, including data generated under this Agreement (Information), the parties agree as follows:

1. Recipient agrees that the Materials, Data, and/or Information will be used for the following research purposes only: to develop future methodologies and to define the most common and relevant loading profiles associated with low back and neck pain, in support of cooperative agreement W81XWH-10-2-0140, "Development of a Novel Translational Model of Vibration Injury to the Spine to Study Acute Injury In Vivo." The Materials, Data, and/or Information shall not be sold, offered for sale, used for commercial purposes, or be furnished to any other party without advance written approval from the Provider's official signing this Agreement or from another official to whom the authority has been delegated, except in accordance with clause 12 of this agreement, and any use or furnishing of Materials and Data shall be subject to the restrictions and obligations imposed by this Agreement. If the Materials, Data, or Information were obtained through human subject research, the Provider acknowledges that they were obtained under an Institutional Review Board (IRB) approved human use protocol that satisfies all scientific and human use review concerns. Likewise, if the Materials, Data, or Information are to be used for human subject and/or animal research, the Recipient assures the Provider that the Recipient will use the Materials/Data/Information under an IRB approved human use or Animal Care and Use Review Office (ACURO) approved animal use protocol that addresses all scientific and human and animal use concerns, respectively.

2. The purpose of this Agreement is the provision of the Materials, Data, and Information as referenced above; no further collaboration is contemplated. Any intellectual property rights to the Materials or Data in existence prior to this Agreement, or potential rights, such as issued patents, patent applications or invention disclosures are retained by the Provider. Any invention patentable under U.S. patent law which is conceived or first reduced to practice under the Agreement shall be owned by the party entitled to ownership under U.S. patent law. The Recipient agrees to grant an exclusive license to any invention arising under this Agreement to which it has ownership to the Provider in accordance with Title 15 U.S. Code Section 3710a, on terms negotiated in good faith. Any invention arising under this Agreement is subject to the retention by the U.S. Government of a nonexclusive, irrevocable, paid-up license to practice, or have practiced, the invention throughout the world by or on behalf of the U.S. Government.

3. The parties shall maintain in confidence all Data and subsequent Information relating to these Materials and Data and shall not disclose Information to others without specific written permission, in advance, unless required to by law. In any event, the parties agree to promptly communicate any third party request for information.

4. When the Materials, Data, or Information are no longer being used for research, in accordance with Item 1 of this Agreement, all Materials and Data will be returned to the Provider. No Materials or Data shall be duplicated for future use or purposes not specifically identified in Item 1 of this agreement.

5. Recipient agrees to report in a timely manner the results of any research with the Material and its products to the Provider. If requested, Recipient agrees to provide all data supporting research results to the Provider.

6. The Materials and Data are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, express or implied. No indemnification for any damages is intended or provided under this Agreement. Each party shall be responsible for any damages it incurs as a result of its activities under this Agreement.

7. Recipient shall accept full responsibility for the safety of the Research Project and assure that the Research Project will be performed in accordance with all Federal, State and local laws, rules and regulations. Where applicable, each party agrees to abide by all laws, rules, and regulations governing biological select agents and toxins.

8. In all oral or written publications concerning the research done or to be done by Recipient with the provided Materials and Data, Provider's contribution is to be expressly noted, by either acknowledgement or co-authorship, as appropriate. For the purpose of restricting any disclosure of Provider's confidential information transferred by the Provider to the Recipient and identified in writing as confidential information by the Provider at the time of transfer, Recipient will send proposed publications to Provider, prior to submission, for review and identification of Provider's confidential information, which the Recipient agrees to remove from the proposed publication. Provider will return comments or suggested revisions to the proposed publications to Recipient within thirty calendar days of their receipt by Provider.

9. If the parties decide to collaborate on research using the Materials, then a new Cooperative Research and Development Agreement will be negotiated which defines the extent of collaboration of the parties.

10. The non-Federal party to this Agreement agrees to make no claim or inference regarding this Agreement, the Materials and Data or their products, which implies governmental endorsement or recommendation.

11. The Materials and Data may not be screened by one or more of the Recipient's laboratories, affiliated laboratories, or contract testing laboratories without written permission of the Provider. The Materials and Data will not be provided to the laboratories of any other entity (industry, academia, other government agency) without permission of the Provider.

12. The construction, validity, performance, and effect of this Agreement shall be governed for all purposes by the laws applicable to the United States Government.

13. The obligation of the parties to transfer technology to one or more other parties, provide technical information and reports to one or more other parties, and otherwise perform under this Agreement are contingent upon compliance with applicable United States export control laws and regulations. Any technical data or commodity transferred from either Party to this agreement to the other Party that may be or is subject to United States export control laws, shall be labeled and identified as such. The receiving Party has the right to refuse acceptance of

such technical data or commodity identified as export controlled. The transfer of certain technical data and commodities may require a license from a cognizant agency of the United States Government or written assurances by the Parties that the Parties shall not export technical data, computer software, or certain commodities to specified foreign countries without prior approval of an appropriate agency of the United States Government. The Parties do not, alone or collectively, represent that a license shall not be required, nor that, if required, it shall be issued. In addition, if applicable, parties to this agreement will comply with 42 CFR section 72 entailing Interstate Shipment of Etiologic Agents.

14. The Provider may terminate this Agreement unilaterally at any time by giving the Recipient written notice.

This Agreement is effective as of the last date of signature of all authorized officials of the parties and shall be effective for three (3) years. This Agreement may be executed in one or more counterparts by the parties by signature of a person having authority to bind the party, which may be by facsimile signature, each of which when executed and delivered, by facsimile transmission, mail, or email delivery, will be an original and all of which will constitute but one and the same Agreement.

UNIVERSITY OF PENNSYLVANIA:

1/6/12
Signature Date

W. STUART WATSON
Associate Director, Office of Research Services

U.S. ARMY AEROMEDICAL RESEARCH LABORATORY:

11 Jan 12
Signature Date

DANA K. RENTA, M.D.
Colonel, Medical Corps, Commander

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UNIVERSITY OF PENNSYLVANIA:

Signature 1/6/12 Date

W. STUART WATSON
Associate Director, Office of Research Services

U.S. ARMY AEROMEDICAL RESEARCH LABORATORY:

Signature 11 Jan 12 Date

DANA K. RENTA, M.D.
Colonel, Medical Corps, Commander

DEPARTMENT OF THE ARMY
U.S. ARMY AEROMEDICAL RESEARCH LABORATORY
FORT RUCKER, AL 36362-0577

MCMR-UAX-SI (70-57) 12 January 2012

MEMORANDUM FOR Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZA-J (Mr. Jurich), Fort Detrick, Maryland 21702-5012

SUBJECT: Cooperative Research and Development Agreement for Material Transfer

1. Forwarded is a Material Transfer Agreement between the U.S. Army Aeromedical Research Laboratory (USAARL) and University of Pennsylvania.

2. The point of contact for this matter is Loraine P. St. Onge, DSN 558-6906.

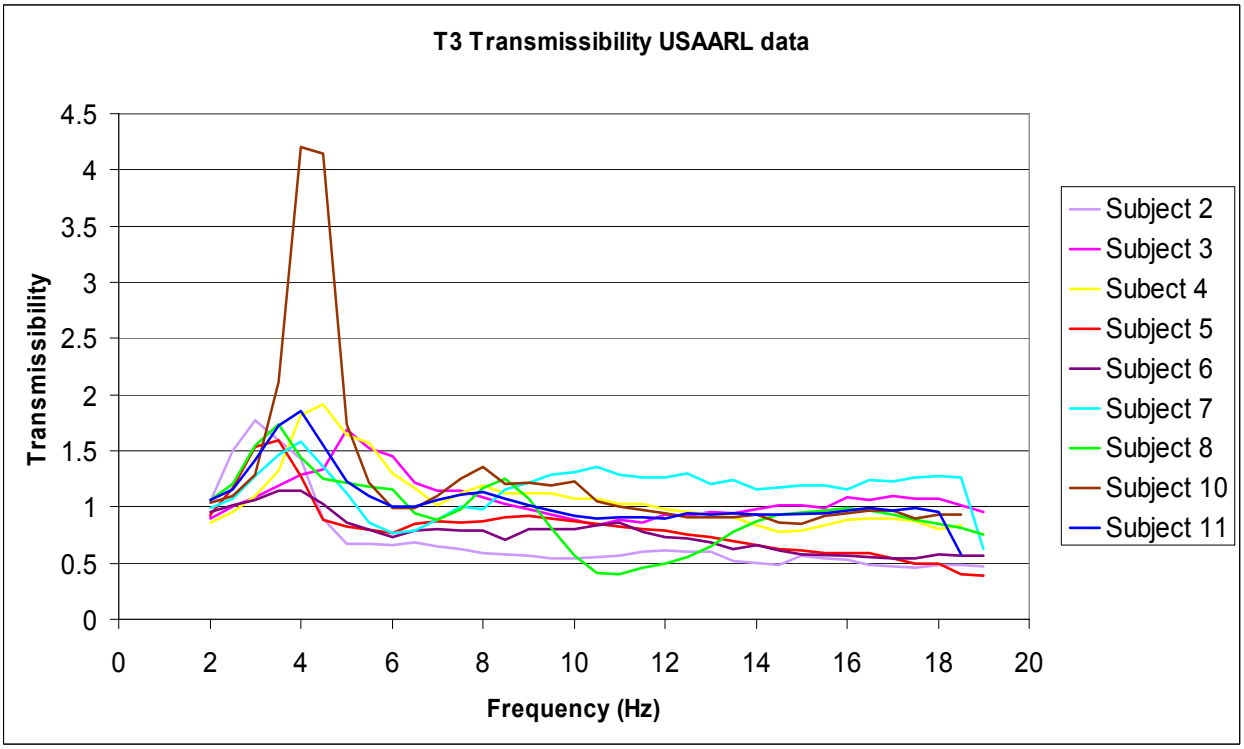
Loraine P. St. Onge

ENCL

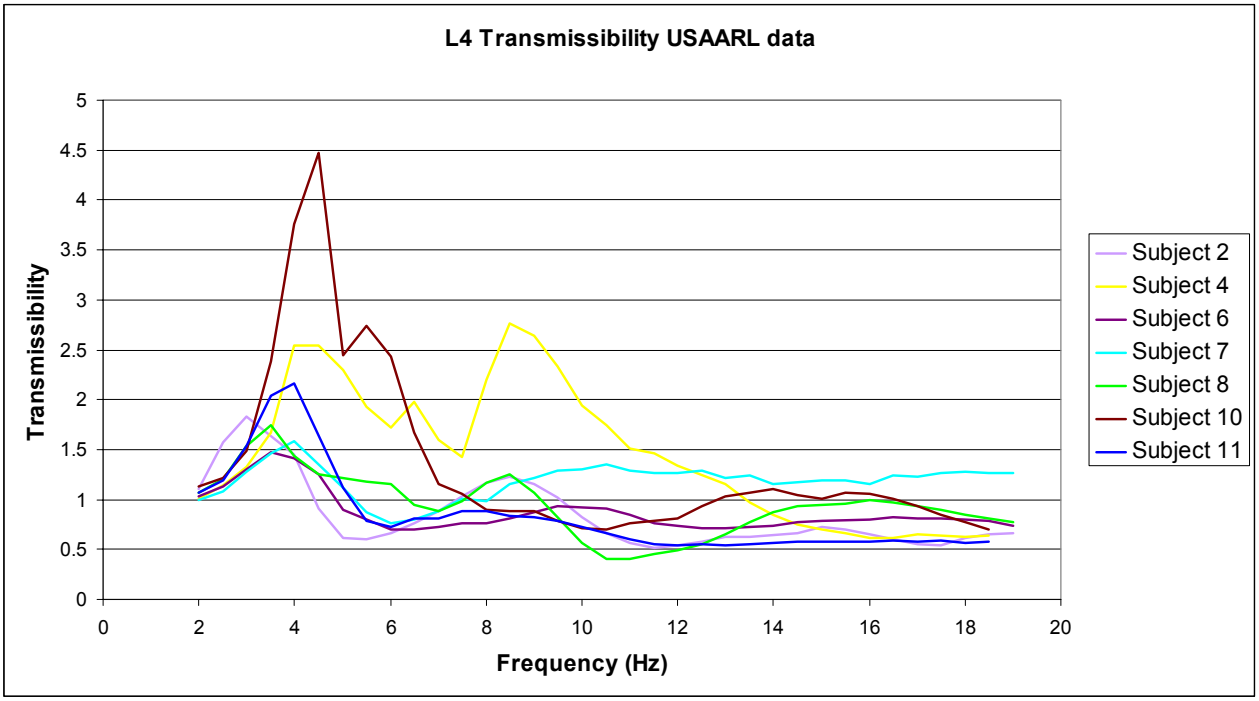
LORAINE P. ST. ONGE
Research Administration Manager
ORTA
USAARL

CF:
COL William Statz
Dr. Carol Chancey
Ms. Bethany Shivers

A2. Vibration Data (Task 2)

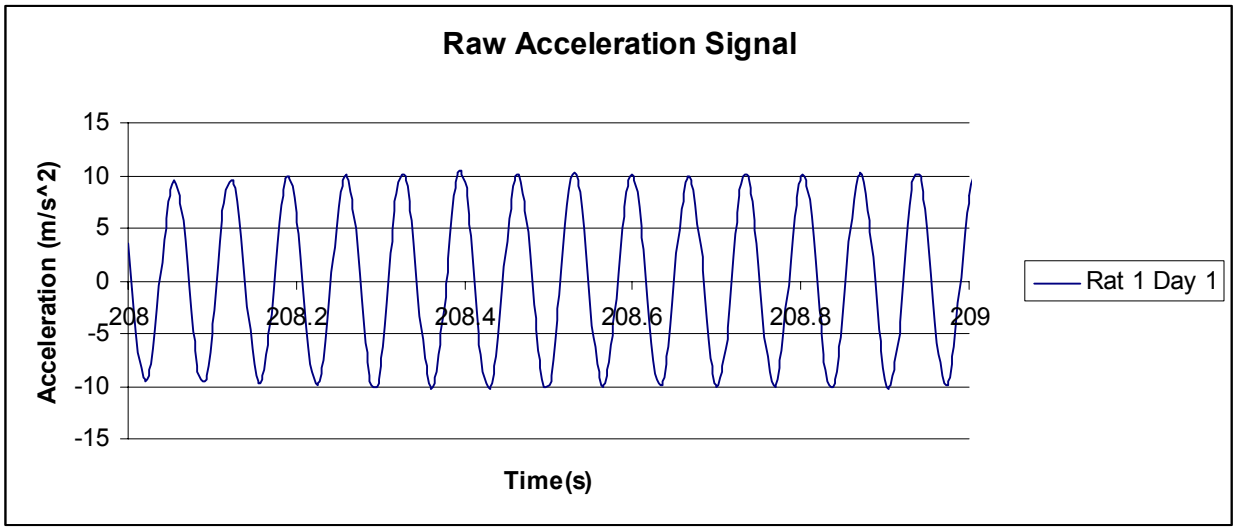


T3 transmissibility response of all subjects (n=9) based on accelerometer data from the seat and T3-mounted accelerometer. Subject #10 exhibited an exaggerated increase in transmissibility at 4 Hz.

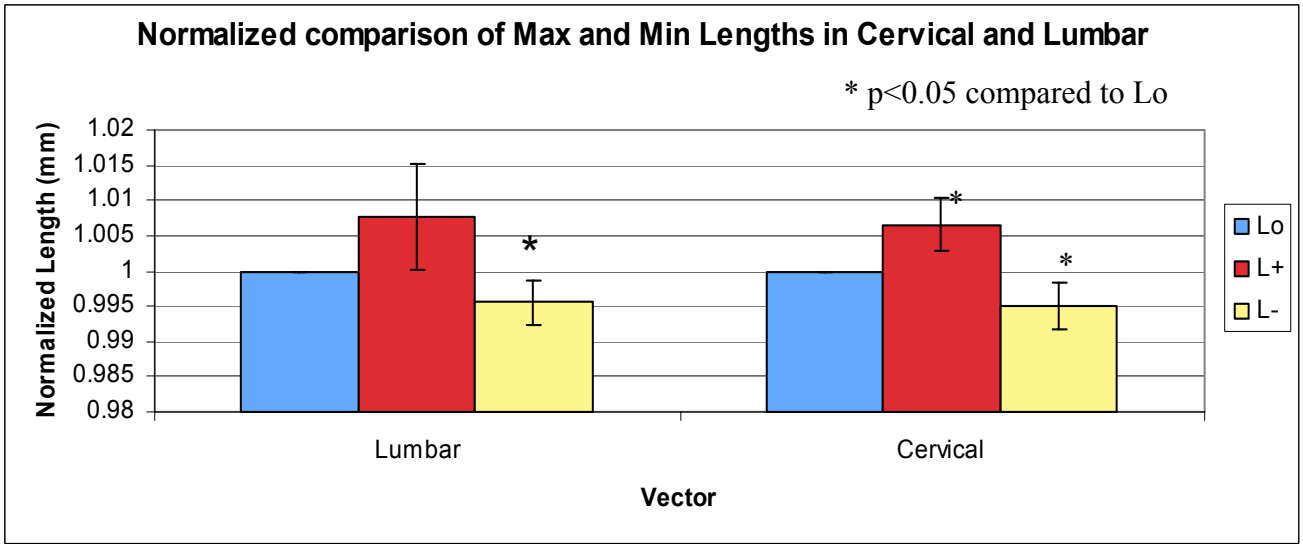


L4 transmissibility response of all subjects (n=9) based on accelerometer data from the seat and L4-mounted accelerometer. Subjects #4 and #10 exhibited an exaggerated increase in transmissibility.

A3. Summary of Acceleration & Compression Data for Studies in Task 3



	Avg Acceleration (m/s^2)	SD	Average Displacement (mm)	SD
Repeated	6.18	0.69	2.01	0.35
Intermittent	6.16	1.01	1.449	0.245
Overall	6.17	0.858	1.66	0.44



A4. Summary of Anatomical Features of Rat Spine (Task 3b)

Cervical measurements (n=5 rats)

	C3	C4	C5	C6	C7	**Average**
VBd/Vd	0.31±0.01	0.35±0.01	0.36±0.02	0.36±0.01	0.36±0.01	**0.35±0.03**
VBw/Vd	0.52±0.03	0.55±0.03	0.57±0.04	0.57±0.04	0.64±0.02	**0.57±0.05**
SCd/Vd	0.47±0.02	0.50±0.02	0.51±0.02	0.49±0.01	0.48±0.02	**0.49±0.02**
SCw/Vd	0.73±0.04	0.85±0.03	0.90±0.02	0.94±0.04	0.92±0.03	**0.87±0.08**
Vw/Vd	1.50±0.06	1.76±0.06	1.88±0.03	2.05±0.07	2.05±0.06	**1.85±0.21**
IFw/Vd	1.25±0.05	1.42±0.03	1.46±0.03	1.44±0.04	1.40±0.06	**1.39±0.09**
VBh/VBd	1.35±0.08	1.24±0.08	1.22±0.10	1.23±0.07	1.30±0.08	**1.27±0.10**
VBw/VBd	1.70±0.12	1.56±0.12	1.58±0.21	1.59±0.08	1.78±0.06	**1.64±0.15**
IVDh/VBd		0.41±0.03	0.35±0.02	0.27±0.01	0.26±0.03	**0.32±0.07**
SCw/SCd	1.55±0.06	1.71±0.07	1.78±0.10	1.91±0.11	1.94±0.13	**1.78±0.17**
Pedicle angle*	51.14±5.48	58.33±6.47	58.67±4.65	61.52±3.54	68.87±2.98	**59.71±7.46**

VB=Vertebral Body; SC=Spinal Canal; V=Vertebra; IF=Interfacet; IVD=Intervertebral Disc
d=depth; w=width; h=height; *Angle measured in degrees

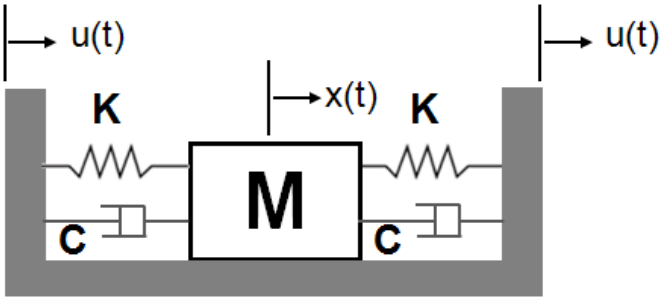
Lumbar measurements (n=5 rats)

	L1	L2	L3	L4	L5	**Average**
VBdu/Vd	0.28±0.02	0.29±0.01	0.28±0.02	0.28±0.02	0.30±0.001	**0.28±0.02**
VBdi/Vd	0.34±0.01	0.35±0.01	0.34±0.01	0.35±0.00	0.34±0.01	**0.34±0.01**
VBwu/Vd	0.43±0.02	0.43±0.01	0.40±0.01	0.38±0.01	0.39±0.02	**0.41±0.03**
VBwi/Vd	0.34±0.01	0.35±0.01	0.34±0.01	0.35±0.00	0.34±0.01	**0.34±0.01**
SCd/Vd	0.35±0.04	0.29±0.06	0.22±0.04	0.16±0.01	0.12±0.02	**0.23±0.09**
SCw/Vd	0.47±0.03	0.42±0.03	0.36±0.03	0.31±0.05	0.24±0.04	**0.36±0.09**
Vw/Vd	0.78±0.44	0.96±0.02	0.70±0.39	0.95±0.03	0.59±0.54	**0.80±0.37**
IFw/Vd	0.71±0.40	0.73±0.41	0.69±0.38	0.58±0.50	0.61±0.34	**0.66±0.36**
VBha/Vbdu	2.22±0.13	2.35±0.08	2.31±0.12	2.11±0.11	1.79±0.21	**2.16±0.24**
VBhp/Vbdu	2.54±0.13	2.71±0.08	2.71±0.19	2.49±0.11	2.12±0.24	**2.51±0.27**
VBha/Vbdi	1.84±0.06	1.95±0.11	1.86±0.06	1.70±0.18	1.55±0.12	**1.78±0.18**
VBhp/Vbdi	2.11±0.13	2.26±0.11	2.18±0.11	2.00±0.19	1.83±0.12	**2.08±0.19**
VBwu/Vbdu	1.55±0.14	1.48±0.04	1.45±0.05	1.34±0.06	1.30±0.08	**1.43±0.12**
VBwi/Vbdi	1.58±0.06	1.57±0.07	1.51±0.08	1.47±0.10	1.41±0.10	**1.51±0.10**
IVDh/Vbdu	0.45±0.14	0.47±0.09	0.48±0.14	0.38±0.24	0.20±0.19	**0.40±0.18**
IVDh/Vbdi	0.45±0.15	0.39±0.08	0.38±0.09	0.39±0.11	0.18±0.16	**0.34±0.13**
SCw/SCd	1.36±0.12	1.52±0.23	1.66±0.17	1.93±0.15	1.96±0.19	**1.69±0.29**
Pedicle angle*	±	±	±	±	±	±

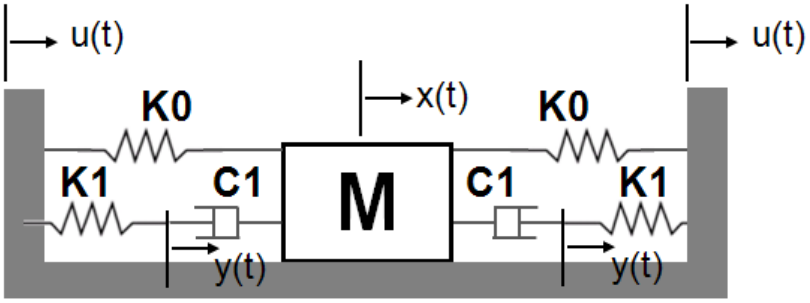
VB=Vertebral Body; SC=Spinal Canal; V=Vertebra; IF=Interfacet; IVD=Intervertebral Disc
d=depth; w=width; h=height; *Angle measured in degrees

A5. 1-DOF Models of Rat under Whole Body Vibration (Task 3g)

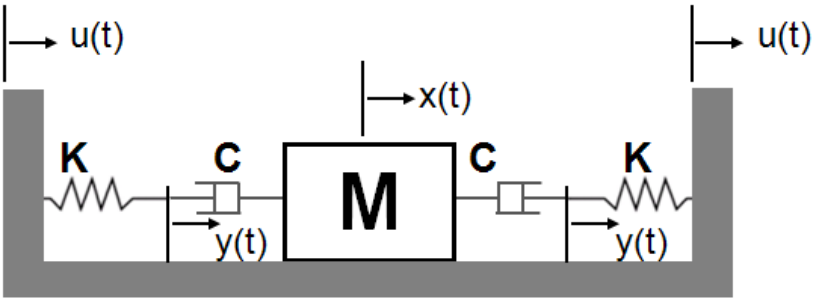
Model 1. Spring and damper in parallel



Model 2. Spring in parallel with elastically coupled damper



Model 3. Spring and damper in series (elastically coupled damper)



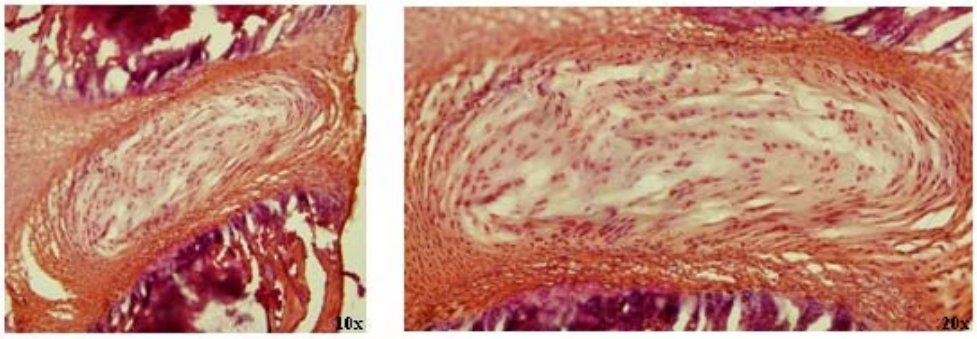
A6. Detailed Inventory of Tissues Harvested to Date (Task 4b)

Summary of Groups at Each Time Point for Whole Body Vibration as of 8/31/2012
Repeated 15Hz (daily exposure for 30 minutes each day, for 7 days)
Intermittent (a single 15Hz exposure on day 0 and day 7)

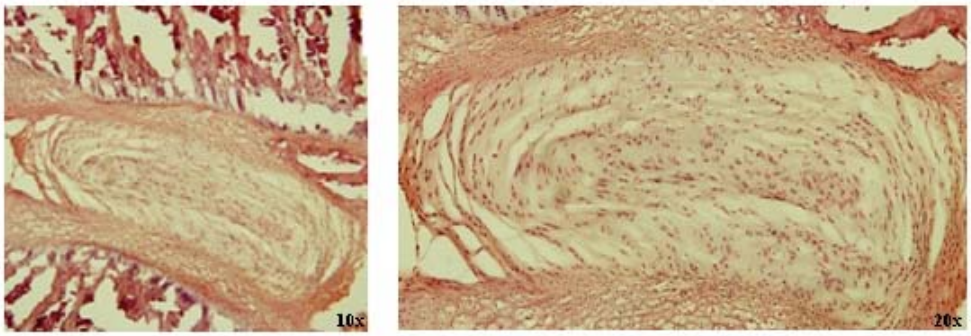
*D1 tissues for repeated and intermittent are from the same animals

Fresh tissue			
Day	Repeated 15Hz	Intermittent	Sham
D14	9	8	8
D8	-	3	-
D7	4	3	-
D1	8	8*	4
Fixed Tissue			
Day	Repeated 15Hz	Intermittent	Sham
D14	4	-	4
D1	4	4*	-

A7. Images of Intervertebral Discs from Spinal Columns



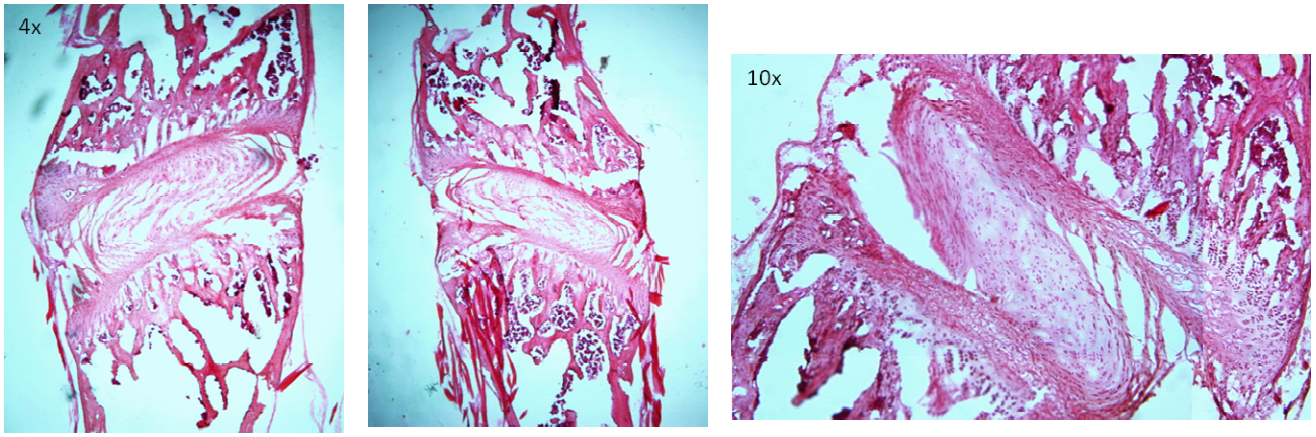
V33, Anesthestia
Sham, Repeated D14
Cervical Disc



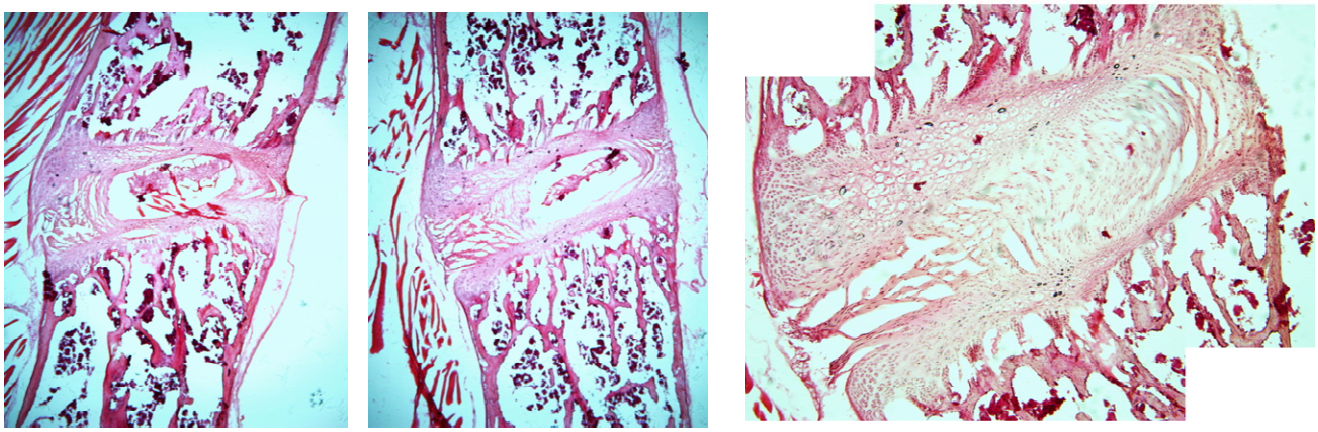
V20, Repeated 15Hz,
D14
Cervical Disc

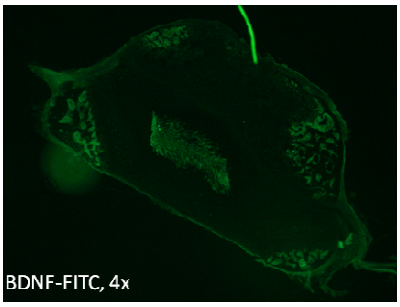
H&E Stain, Cervical Spinal Column: Sectioned (4/13/2012), Stained (9/14/2012)

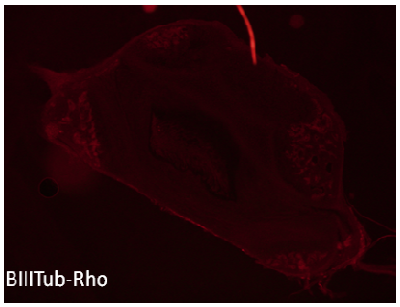
Vibration, 15 Hz Repeated, D14

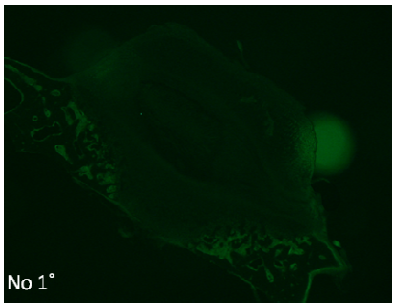


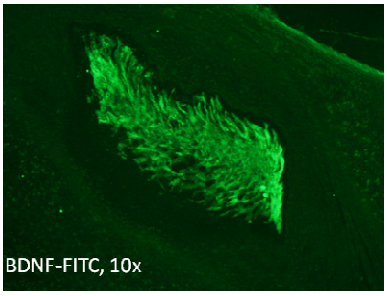
Anesthesia/Sham, D14

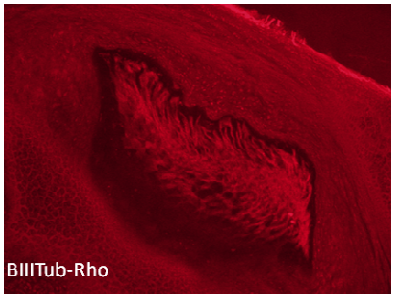


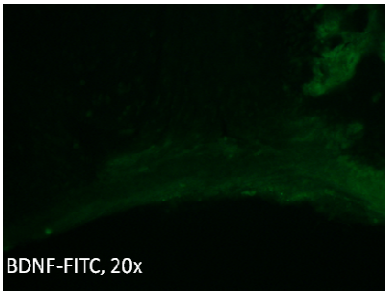


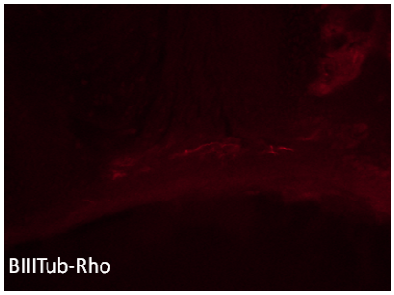




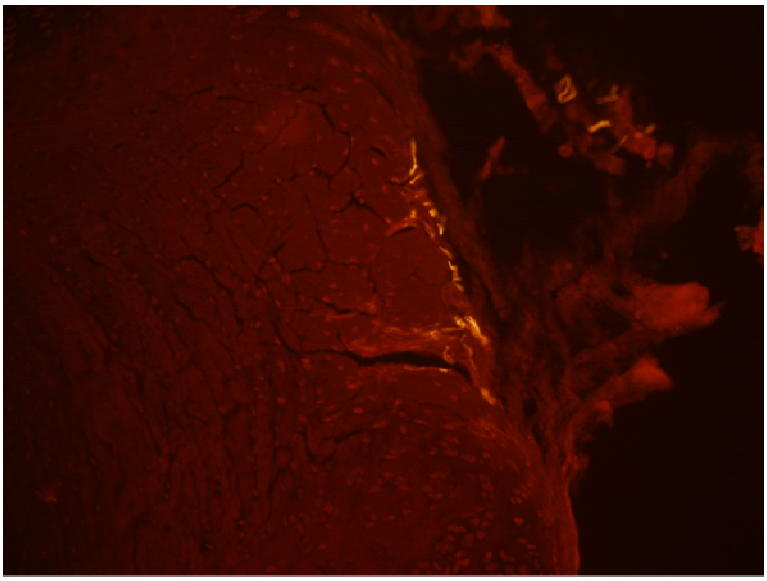




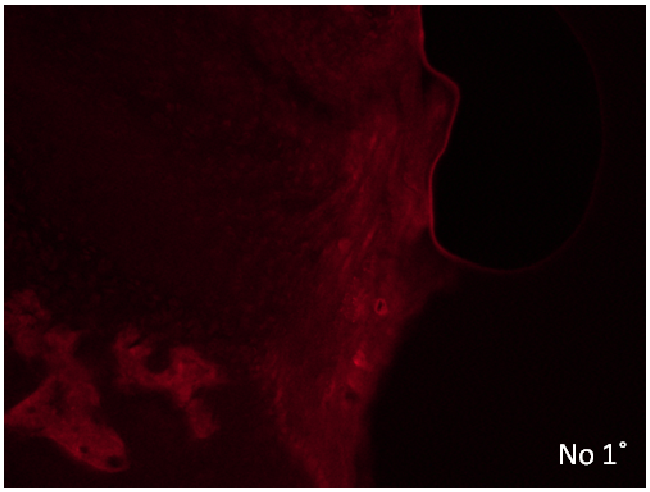


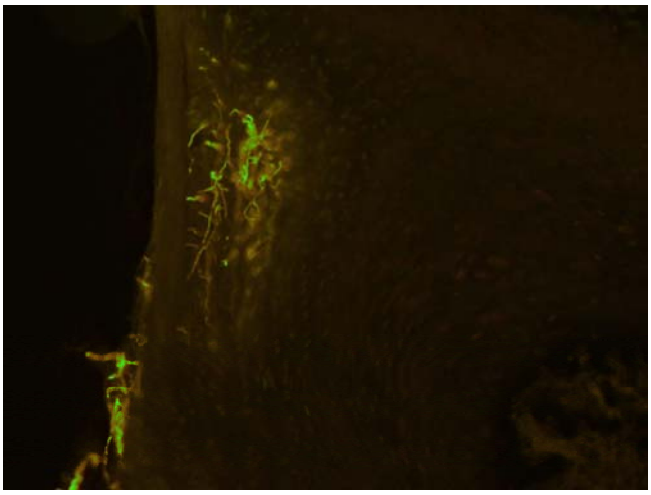


BDNF (FITC) and BetaIIITub (Rhodamine), separate channels. Cervical Disc, Rat 18 sham

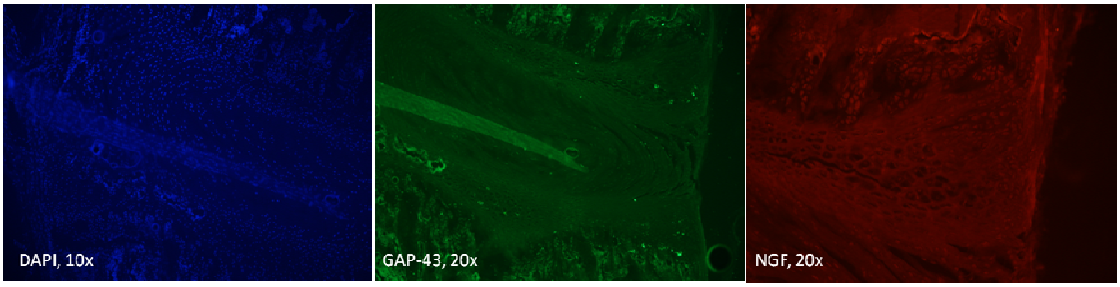


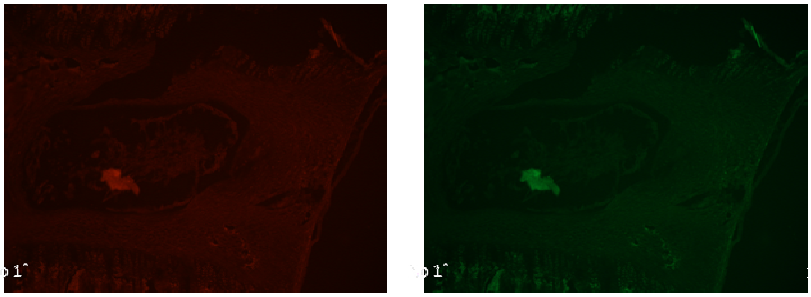
Rat 21, vibrated, cervical, 20x βIIITub





NGF (FITC) and BetaIIITub (Rhodamine), merged
Cervical Disc, Rat 18 sham





GAP-43 (FITC) and NGF (Rhodamine), separate channel, Cervical Disc, Rat 20, 15 Hz Repeated D14

A8. Abstracts published in the last Year (Task 5)

Abstract Presented at Northeast Bioengineering Conference in March, 2012.

Repeated Whole-Body Vibration Exposure Induces Prolonged Mechanical Hyperalgesia & Increased Spinal COX-2: A Novel Rat Model

B.B. Guarino, H.A. Baig, J.L. Branconi, B.A. Winkelstein
University of Pennsylvania
210 S 33rd Street
Philadelphia, PA 19104

Abstract **– Whole-body vibration (WBV) has been linked to chronic back pain but the mechanisms of its development are unknown, as is how biomechanical contributions modulate the pain response. An animal model of WBV was developed in which rats were exposed to 7 days of either 5Hz or 15Hz vibration. WBV induced immediate sensitivity (i.e. pain) that was sustained for 7 days after the cessation of the WBV exposure. There was no difference in the pain response between the two vibration frequencies. Increases in the inflammatory mediator, COX-2, were also induced following painful WBV in the lumbar spinal cord, suggesting a potential mechanism for the chronic pain.**

I. Introduction

Repeated exposure to whole-body vibration (WBV) is thought to lead to chronic back pain [1]. Limited studies have investigated the neurophysiologic and mechanical effects of WBV exposure [2,3]. Changes in several neuropeptides related to nociception (substance P and VIP) were observed in the lumbar dorsal root ganglia and the resonance frequency of the rodent was estimated to be between 3.5 and 5Hz [2]. While studies strongly suggest WBV as a potential mechanism to induce pain, and provide important mechanical context for that hypothesis, the relationship to pain still remains speculative.

The goal of this study was to develop a model of WBV in the rat, and to investigate the effect of vibration frequency on pain symptoms and a potent nociceptive mediator in the spinal cord. Based on prior animal and transmissibility studies, two vibration frequencies were used [2,3]: 5Hz and 15Hz. Rats were monitored for behavioral sensitivity (i.e. pain) during and after the WBV exposure period. COX-2 was also assayed in the spinal cord since it is a known spinal inflammatory mediator of pain [4].

II. Methods

All procedures used male Holtzman rats (275-325g), were IACUC-approved, and adhered to the guidelines for Research and Ethical Issues of the IASP.

A. Whole-Body Vibration Exposure

Vibration exposure was performed under inhalation anesthesia (4% isoflurane for induction, 3.5% for maintenance). Whole-body vibration was applied daily for a period of 30 minutes on 7 consecutive days. During each WBV exposure session, the rat was placed in a prone position and secured to a customized acrylic platform by velcro straps (Fig. 1). The platform was rigidly fixed to a linear servomotor (MX80L; Parker Hannefin) that was programmed and controlled by a digital driver (VIX500IH; Parker Hannefin) to translate the platform through a full stroke distance of 1.5mm. A laser LVDT (LTC–050-10; MTI) also tracked the platform motion. Two miniature quartz shear accelerometers (ACC104A; Omega) quantified accelerations of the plate and the rat; one accelerometer was affixed to the plate and the other was embedded in a velcro strap secured to the rat near the lower thoracic and upper lumbar spines (Fig. 1).

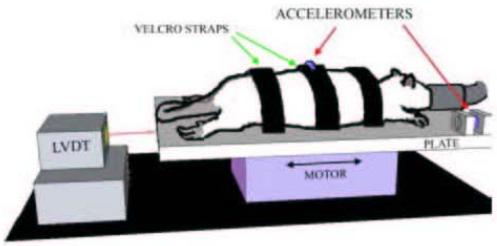


Fig. 1. Schematic of WBV device, indicating the set-up and instrumentation.

Separate groups of rats were exposed to either a 5Hz vibration (n=3) or a 15Hz vibration (n=7) during each WBV session. A sham control group (n=4) received only anesthesia treatment matching the dose and timing of the WBV groups.

B. Behavioral Assessments

Behavioral sensitivity was assessed by measuring mechanical hyperalgesia in both of the forepaws and hind paws [5]. Hyperalgesia was measured prior to (day 0) the first day of WBV exposure and daily on the morning following the prior day's WBV session. Response thresholds were also measured for an additional 7 days after the cessation of the WBV exposures. For each session, the plantar surface of each paw was stimulated with a range of von Frey filaments (0.4–26g) using customary methods [5]. The average threshold was taken as the threshold for each paw, day, and group. A repeated-measures ANOVA with post hoc Bonferroni compared response thresholds between groups over time.

C. Western Blot of COX-2 in Spinal Cord

Spinal cord tissue was harvested on day 14 to quantify COX-2 protein using western blot analysis. The cervical and

lumbar enlargements were separately isolated and whole protein lysates obtained. Protein (1.35µg/µl in 37µl) was loaded into a polyacrylamide gel for electrophoresis and transferred to a PVDF membrane (Millipore). The membrane was incubated with polyclonal rabbit anti-COX-2 (1:200; Abcam) and rabbit anti-actin (1:200; Santa Cruz) antibodies, followed by an 800nm goat anti-rabbit antibody (1:15,000; LI-COR). The membrane was imaged and analyzed by an Odyssey infrared fluorescence detector system (LI-COR). COX-2 expression for each sample was normalized by actin levels and compared between WBV and sham using separate t-tests for each of the cervical and lumbar spinal cords.

III. Results

Response thresholds for both the forepaw and hind paw were reduced immediately and continually decreased for both 5Hz and 15Hz WBV (Fig. 2). In contrast, sham controls did not exhibit any change from their responses prior to testing (day 0) (Fig. 2). Overall, both forepaw and hind paw sensitivity was significantly increased for both 5Hz ($p<0.001$) and 15Hz ($p<0.001$) (Fig. 2). During the period of daily WBV exposure, withdrawal thresholds significantly decreased for both 5Hz (forepaw $p<0.001$; hind paw $p=0.036$) and 15Hz (forepaw $p<0.001$; hind paw $p<0.001$). This relationship persisted after WBV, for both 5Hz (forepaw $p=0.002$; hind paw $p=0.001$) and 15Hz WBV (forepaw $p<0.001$; hind paw $p<0.001$) (Fig. 2). There was no difference in sensitivity between the 5Hz and 15Hz WBV groups at any time point.

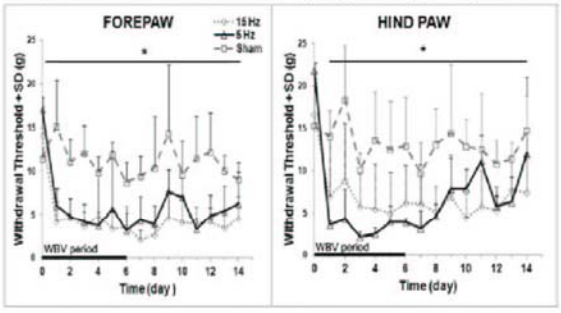


Fig. 2. Withdrawal thresholds for WBV (15Hz, 5Hz) and sham groups in the forepaw and hind paw.

As with the behavioral responses (Fig. 2), no differences were detected between COX-2 expression for 5Hz and 15Hz WBV, in either of the cervical or lumbar spinal cords. As such, samples from those groups were pooled to compare COX-2 expression between WBV and sham. COX-2 was not changed in the cervical spinal cord after WBV (Fig. 3). In contrast, lumbar COX-2 expression after WBV was nearly twice that of sham levels; this increase was significant ($p=0.041$) (Fig. 3).

IV. Discussion

This study is the first to demonstrate that repeated WBV exposure induces prolonged mechanical hyperalgesia in rats. Although it has been shown that frequency affects both the apparent mass and transmissibility in seated human subjects [3], no changes in pain or COX-2 levels were noted between the two vibration frequencies used in this study (Figs. 2 & 3). But, the increase in spinal COX-2 in association with sustained pain is consistent with other reports and suggests WBV may induce some form of neural injury in WBV [4].

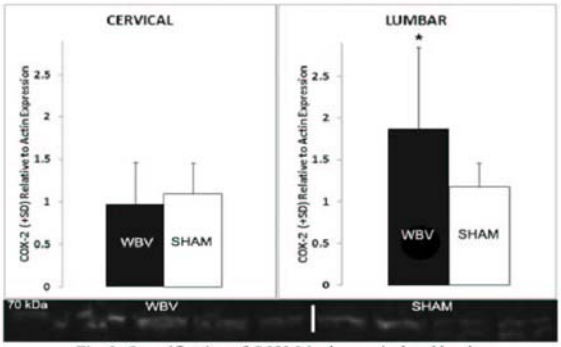


Fig. 3. Quantification of COX-2 in the cervical and lumbar spinal cord after WBV and sham. Lumbar COX-2 significantly increased in WBV.

Although the applied acceleration of the rats during WBV in this study was aligned along the long-axis of the spine, this does not fully simulate a vertical spinal vibration that is common for human exposure. While the increase in spinal COX-2 suggests WBV may injure spinal and/or peripheral tissues, this study did not identify the source of such modifications. Studies assaying neuroinflammatory responses in muscle, disc, and other tissues will provide insight about this type of painful injury. Nonetheless, these result provide an important foundation for understanding painful WBV.

Acknowledgment

This work was supported by a grant from the Department of Defense (W81XWH-10-2-0140).

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Abstract Presented at Northeast Bioengineering Conference in March, 2012.

The Transmissibility Response of the Rat During Whole Body Vibration Along its Long-Axis

H.A. Baig, B.B. Guarino, N.V. Jaumard, B.A. Winkelstein
University of Pennsylvania
210 S 33rd Street
Philadelphia, PA 19104

Abstract **– Vibration exposure has been suggested as a painful injury mechanism, but the relationship between mechanical loading and pain is unknown. The rat provides a useful model system for such pain studies, but the vibration response of the rat has not been studied. Moreover, in vivo systems preclude the use of implantable transducers needed to measure accelerations. This study used image-based and accelerometer-based methods to determine the transmissibility response of the rat for whole body vibration. Resonance was found at 8 Hz, with no difference in the responses using either image or accelerometer analyses.**

I. Introduction

Vibration exposure has been linked to chronic pain and low back pain symptoms [1]. Only a few studies have investigated the mechanical effects of whole body vibration using human and primate subjects [2,3]. Moreover, although the rat provides a desirable model system for studying pain and neurophysiology responses related to tissue loading and injury [4], there is still very little known about its mechanical response to vibration. Although the dynamic response of other species has been defined [3,5], the transmissibility of the rat's spinal column and/or its response to whole body vibration along its long-axis is unknown.

Because of the growing use of the rat as a platform to model pain and tissue injury, the goal of this study was to characterize the frequency response of the rat during whole body vibration along its long-axis and to determine its resonant frequency. In addition, transmissibility was measured using a direct method of accelerometers as well as an indirect image-based approach and the two methods were compared.

II. Methods

A. Experimental Setup & Data Acquisition

Rats underwent whole body vibration when in the prone position, with the primary direction of vibration along the spine's long-axis, using IACUC-approved procedures. Male Holtzman rats (310-350g; n=5) were secured to an acrylic base plate using two velcro straps (Fig. 1). The base plate was coupled to a linear servo motor that was controlled by a driver (MX80L & VIX500IH; Parker Hannifin) and imposed a 1.5 mm peak-to-peak displacement of the plate. Transmissibility was computed using the input and output accelerations of the system. The input acceleration was measured using an accelerometer mounted on the base plate; the output acceleration was measured using an accelerometer affixed to the lumbar region of the rat by a strap (Fig. 1). Both DC accelerometers have a range of ±500Gs and a sensitivity of 10mV/G (Model 7521A; Dytran). Markers were placed on the base plate and the lumbar accelerometer and were tracked by a high speed CCD camera (VRI-MIROEX1-1024MM; Phantom; 640X480) during vibration (Fig. 1). These markers were used to derive the corresponding input and output motions of the system

Two studies were performed to calculate the transmissibility of the rat in different conditions, and using accelerometer and image-derived data. In the first study, euthanized rats (n=5) were exposed to sinusoidal whole body vibrations at frequencies ranging from 3 Hz to 15 Hz. For each frequency magnitude, the rat underwent 60 seconds of vibration, followed by a rest period of 120 seconds before being exposed to the next highest frequency. Accelerometer and image data were recorded during each frequency exposure at a sampling rate of 120 Hz. In the second study, anesthetized rats were similarly vibrated at either 5 Hz (n=4) or 15 Hz (n=4) and image data were acquired in the same way as described above.

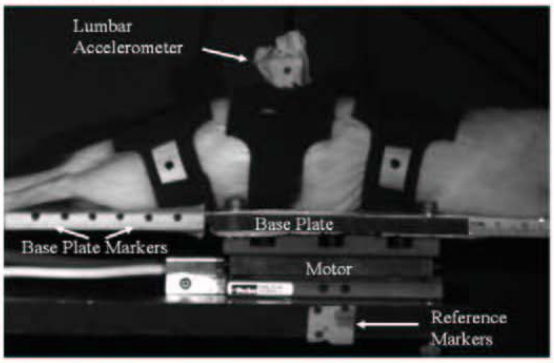

Fig. 1. Experimental set up imaged using CCD camera showing base plate, motor, lumbar accelerometer, and markers.

B. Analysis of Acceleration Data

For all analyses, 30 seconds of accelerometer data and 12 seconds of video data were used. The marker displacements were determined from the images by digitizing their positions relative to stationary reference markers in each image frame using ProAnalyst software. The corresponding input and output displacements were doubly differentiated to derive the corresponding input and output accelerations. Both image and accelerometer acceleration data were filtered using a 5th order

Butterworth bandwidth filter. The transmissibility of the system for each vibration condition was calculated as the ratio of the root mean square of the output acceleration to the root mean square of the input acceleration [2]. The acceleration signals were transformed into the frequency domain; then the angle of the input acceleration was subtracted from the angle of the output acceleration in order to calculate the phase shift at each frequency. In the first study, the transmissibility and phase shift were determined using both the accelerometer and image data and were compared using an F-test. Similarly, using the image data from the second study, an F-test was also used to determine if there is any difference in the transmissibility response compared to an anesthetized rat in this test system.

III. Results

The resonance frequency of a rat in this whole body vibration system was determined to be 8 Hz (Figs. 2 & 3). Further, the transmissibility response was the same whether determined by image or accelerometer data, with no significant difference ($F=0.073$; $F_c=2.82$) (Fig. 2). The transmissibility response was similar at the extremes of frequencies tested, with a value of 0.94±0.12 at 3 Hz and 0.67+0.04 at 15 Hz (Fig. 2). Also, the phase shift decreased with increasing frequency, with an abrupt drop in phase shift at 8 Hz (Fig. 3), corresponding to the resonant frequency of 8 Hz determined by the transmissibility response (Fig. 2).

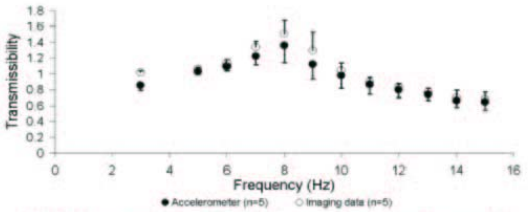

Fig. 2. Transmissibility calculated from accelerometer and image data

Interestingly, there was no significant difference between the transmissibility determined using the image data from either the anesthetized rats or the expired rats at either 5 Hz ($F=0.0104$; $F_c=9.12$) or 15 Hz ($F=4.12$; $F_c=9.12$).

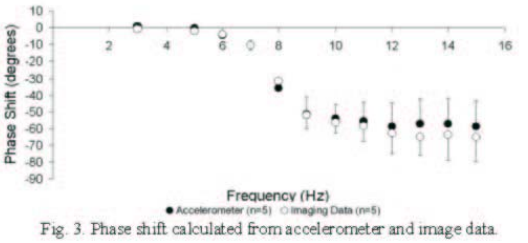

Fig. 3. Phase shift calculated from accelerometer and image data.

IV. Discussion

This study determined the resonant frequency of the rat to be 8 Hz (Figs. 2 & 3), which is similar to other studies with different species. The resonant frequency of the both the human and rabbit has been approximated to be at 4.5 Hz [5,6]; the primate has been shown to be in the range of 9–15 Hz [3]. However, for those studies, the primates and humans were vibrated in the seated position and transmissibility was measured along the axis of the spine [3,6]. The rabbit study was performed using a set-up similar to that used in the current study [5]. Yet, our study finds a similar response, and supports the use of a non-invasive imaging approach

The transmissibility was found to be same at the higher and lower ends of the frequencies tested here (Fig. 2) and may suggest that similar injuries may be induced regardless of vibration frequency. Yet, additional studies are needed to understand the relationship between the mechanical response of this system and the associated physiological correlates. While this study did not investigate the response in a living system, the similarities at 5 and 15 Hz in the two studies suggests the results of the expired rats may likely translate to an anesthetized rat. Even the anesthetized response does not incorporate the effects of musculature due to their absence under anesthesia. The acceleration magnitude was not held constant and it is not known how responses are changed for different acceleration profiles. In fact, an increase in acceleration has been shown to decrease the resonance frequency in human studies [2]. In contrast, the response at different accelerations was not different in a study of spinal vibration using a transducer that was directly attached to the spinous processes in the lumbar spine [6]. Certainly, continued studies under different vibration conditions and incorporating assays of tissue mechanics, physiology and function will improve our understanding of this system and its response to a common biomechanical injury mode.

Acknowledgment

This work was supported by a grant from the Department of Defense (W81XWH-10-2-0140).

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Abstract Presented at Northeast Bioengineering Conference in March, 2012.

The Rat as a Viable Model for Human Cervical Biomechanics: A Quantitative Anatomy Study

Akhilesh J. Gokhale*, Benjamin B. Guarino, Beth A. Winkelstein
Departments of Bioengineering and *Mechanical Engineering
University of Pennsylvania, Philadelphia, PA

Background

- Many species, such as the sheep, goat, pig, calf, dog and rat are commonly used as a model for the human spine to study spinal injury biomechanics [1-4].
- With the increase in rat modeling for injury biomechanics, it is necessary to characterize the bony anatomy of the spine of that species in order to evaluate it as a potential model system mimicking human injury.
- Although several studies have performed such analyses for the lumbar spine [2,3], corresponding comparative data for the cervical spine is still lacking.

The objective of this study was to quantify the cervical bony anatomy in the rat using microCT and compare it to the human spine.

Materials & Methods

MicroCT Imaging and Processing :

- The cervical spine from C3-C7 was removed from male rats (n=3; 314-348g).
- Spines were scanned using a microtomographic system (vivaCT 40; Scanco) in multi-slice mode at a slice thickness of 0.38μm and an axial field of view of 1024x1024, with 32-bit-gray levels.
- MicroCT data were analyzed in ITK-SNAP software to segment the bony structures using the gray-level intensity threshold. Individual vertebrae were identified, delineated, and reconstructed in ITK-SNAP to render the cervical spine in three-dimensions (3D) (Figs. 1 & 2).

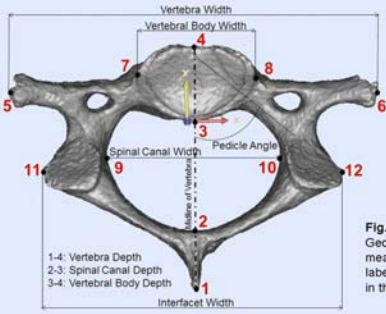

Fig. 1. Geometrical measurements labeling the vertebra in the axial plane.

Quantitative Anatomical Measurement:

- 3-matic Software (Materialise) was used to quantify several measurements of the bony anatomy. The local coordinate system of each vertebra was aligned with the global coordinate system of the image-space (Fig. 1).
- Several anatomical parameters were used to quantify bony characteristics (Table 1):

Table 1. Anatomical Parameters and their Nomenclature (Refer to Figs. 1 & 2)

ID	Description	Points	Plane
Vd	Maximum vertebra length along anteroposterior direction	1-2	Axial Plane
VBd	Maximum vertebral body length along anteroposterior direction	3-4	Axial Plane
SCd	Maximum spinal canal length along anteroposterior direction	2-3	Axial Plane
Vw	Maximum lateral dimension of vertebra normal to midline	5-6	Axial Plane
VBw	Maximum lateral dimension of vertebral body normal to midline	7-8	Axial Plane
SCw	Maximum lateral dimension of spinal canal normal to midline	9-10	Axial Plane
IFw	Maximum distance between articular masses normal to the midline	11-12	Axial Plane
VBh	Vertebral body length from superior aspect of upper endplate to inferior aspect of lower endplate measured at the anterior edge of each vertebra.	13-14	Coronal Plane
IVDh	Intervertebral disc height from inferior aspect of corresponding upper endplate to superior aspect of lower endplate	14-15	Coronal Plane
Pedicle angle	Angle between midline of pedicle and vertebral body		Axial Plane

- To account for differences in measurements due to specimen variability, all width measurements were normalized by vertebra depth at each level and all height measurements were normalized by the corresponding vertebral body depth.
- Measurements were made on each vertebra five times to quantify repeatability.

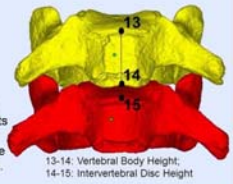

Fig. 2. Geometrical measurements labeling the vertebra in the coronal plane.

Results

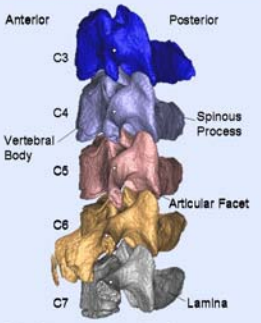

Fig. 3. Lateral view showing 3D reconstruction of cervical spine from C3-C7.

- The quantification method is very repeatable, with variability less than 0.2mm for linear measurements (corresponding to 0.06 in the normalized anatomy ratios) and less than 3° for angular measurements.
- All vertebrae are wider than deep (1.5-fold at C3 & 2-fold at C7)
- The vertebral body is wider and taller than deep (Figs. 1 & 3). Its width is 1.6 times its depth and 1.3 times its height (Table 2).
- The spinal canal is elliptically shaped, becoming wider in the transverse direction in the lower cervical spine (Table 2).
- The height of the intervertebral disc decreases by nearly 30% from C4-C5 (0.35) to C5-C6 (0.27) (Table 2).
- The interfacet pedicle angle increases in the lower cervical spine, to a maximum of nearly 70° at C7, indicating a more broadly splayed facet angle in the lower cervical spine.

Table 2. Average Anatomical Measurements By Level & Overall

	C3	C4	C5	C6	C7	Average
VBd/Vd	0.32±0.01	0.36±0.01	0.37±0.03	0.36±0.01	0.37±0.01	**0.35±0.02**
VBw/Vd	0.52±0.03	0.53±0.01	0.57±0.05	0.57±0.04	0.65±0.01	**0.57±0.06**
SCd/Vd	0.47±0.02	0.49±0.02	0.50±0.02	0.49±0.02	0.47±0.02	**0.48±0.02**
SCw/Vd	0.74±0.05	0.84±0.03	0.90±0.03	0.94±0.05	0.92±0.04	**0.87±0.08**
Vw/Vd	1.52±0.04	1.72±0.02	1.88±0.01	2.03±0.07	2.03±0.07	**1.84±0.20**
IFw/Vd	1.27±0.05	1.42±0.03	1.47±0.03	1.45±0.05	1.38±0.05	**1.40±0.08**
VBh/VBd	1.31±0.08	1.20±0.07	1.19±0.12	1.21±0.08	1.28±0.03	**1.24±0.09**
VBw/VBd	1.63±0.08	1.48±0.04	1.58±0.24	1.60±0.09	1.79±0.05	**1.62±0.16**
IVDh/VBd		0.41±0.03	0.35±0.02	0.27±0.01	0.26±0.03	**0.32±0.07**
SCw/SCd	1.58±0.05	1.73±0.09	1.79±0.12	1.93±0.14	1.97±0.12	**1.80±0.18**
Pedicle angle*	47.89±3.04	53.41±2.88	55.03±1.02	59.10±1.55	68.91±3.10	**56.87±7.44**

VB=Vertebral Body; SC=Spinal Canal; V=Vertebra; IF=Interfacet; IVD=Intervertebral Disc
d=depth; w=width; h=height; *Angle measured in degrees

Discussion

- By normalizing the measurements of width and height by depth, comparisons of these measurements can be made between the rat and human cervical spines. Accordingly, the rat vertebral body is characteristically taller than deep, by a factor of 1.24 (Table 2), whereas the human has a ratio of 0.84 [5,6], suggesting the rat's cervical spine may be more flexible in the sagittal plane than the human's. This is an important factor when considering the different modes of loading and biomechanical considerations of simulating neck injuries in the rat.
- In contrast, the vertebral body width (0.57) and depth (0.35) ratio measurements in the rat are fairly consistent with the human (0.56, 0.34, respectively) [7,8].
- The spinal canal width in the two species is also nearly identical. In the rat the increase in width is nearly 25% (1.58 at C3 to 1.97at C7; Table 1) compared to the 15% increase in the human (from 1.45 to 1.68) [8-10].
- Although these results are based on only a small number of rats (n=3), the resolution and consistency of measurements strengthen its validity.
- This study of the cervical spine supports prior similar reports for the lumbar spine [1,4] – that the rat may be a viable model for some particular biomechanical studies. Our data also highlight potential differences in the geometry of the rat's cervical spine that may be important for certain modes of loading, such as bending and axial compression, where the neck's column slenderness ratio is an important factor in the spine's structural biomechanics.

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Acknowledgments

This work was supported by a grant from the Department of Defense (W81XWH-10-2-0140).

Abstract Presented at Northeast Bioengineering Conference in March, 2012.

Painful Whole Body Vibration Increases NGF and BDNF in Cervical Intervertebral Disc in the Rat

J.L. Branconi, B.B. Guarino, H.A. Baig, B.A. Winkelstein
Spine Pain Research Lab
Department of Bioengineering
University of Pennsylvania, Philadelphia, PA

Background

- Chronic neck pain affects between 12–71% of the adult population, and imposes high financial burdens, with annual costs over $29 billion.[1,2]
- Repeated spinal loading, as can occur from whole body vibration (WBV), is linked to pain and intervertebral disc degeneration.[3-4]
- Although studies have related low back pain to pathology in the lumbar disc from its mechanical loading,[5] the effects of similar repeated loading or spinal vibration have not been defined for the cervical spine and/or its discs.
- Increasing evidence shows an upregulation of neurotrophic factors and concomitant growth of nerve fibers into otherwise aneural regions in painful and degenerate lumbar discs.[6]

Study Objective: The goal of this study was to investigate the effects of painful WBV on the expression of two neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), in cervical intervertebral discs following vibration.

Materials & Methods

Vibration Procedures & Behavioral Assessments:

All procedures were IACUC-approved. Male Holtzman rats (250-275 gm) were exposed to WBV (n=6) under anesthesia. For each WBV session, the rat was placed on a platform that was vibrated at 15 Hz over 1.5 mm for 30 minutes (Fig. 1); this WBV was performed daily for 7 days. Sham rats (n=4) were also included in which anesthesia was given under the same daily protocol; no vibration was applied.

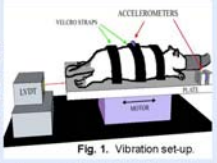

Fig. 1. Vibration set-up.

Behavioral sensitivity was assessed as an indication of pain; the threshold for paw withdrawal was measured in both forepaws.[7] Testing occurred prior to the first exposure and on days 7 and 14, and the percent threshold change from baseline was determined. Mean changes in response threshold were calculated for each group at each day and compared using a one-way ANOVA with a post-hoc Bonferroni-Holm test.

Western Blotting for Detection of NGF & BDNF:

On day 14, cervical spinal columns were harvested. The cervical discs were excised and immediately placed on dry ice. Whole protein lysates were obtained, separated by molecular weight, transferred to a PVDF membrane for immunolabeling as described previously.[8] Individual bands of interest were measured; separately, the mean integrated intensity of NGF and BDNF was normalized to the mean integrated intensity of GAPDH. Intensities were averaged for each group. Differences in the proteins were detected between the WBV and sham groups using separate t-tests for each of NGF and BDNF.

Results

- WBV produced a significant reduction in the threshold stimulus to elicit a paw withdrawal at both day 7 ($p<0.001$) and day 14 ($p<0.001$) (Fig. 2). However, the responses of the unvibrated sham control rats remained unchanged from baseline at both days. Sham rats exhibited elevated thresholds over WBV rats at both time points ($p=0.004$ day 7; $p=0.016$ day 14) (Fig. 2).
- Both NGF and BDNF were detected in all of the disc lysates (Fig. 3) and the levels of each protein increased following WBV (Table 1).
- NGF increased nearly 5-fold, depending on the molecular weight isoform that was probed (Table 1). The proNGF form increased the most (4.9±0.01 fold over sham), though the increase did not reach significance ($p=0.055$). A high molecular weight isoform of NGF nearly doubled and this increase was significant ($p=0.03$) (Table 1).
- BDNF levels also increased significantly ($p=0.0006$) over sham after WBV (Fig. 3; Table 1).

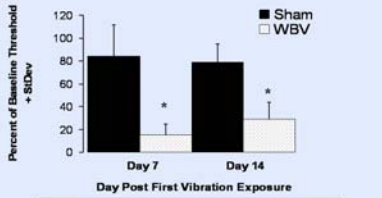

Fig. 2. Forepaw hyperalgesia (i.e. a decreased threshold) is induced early after WBV (day 7; *p=0.004) and sustained for 7 days following that (day 14; *p=0.016).

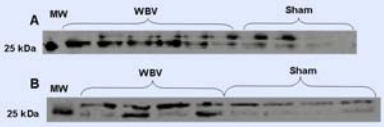

Fig. 3. Both NGF (A) and BDNF (B) increase in the disc following WBV.

TABLE 1
FOLD-CHANGES IN GROWTH FACTORS OVER SHAM

WBV	n	Fold increase over sham	Standard Deviation	p value
NGF 75 kDa	6	1.8	0.01	0.03
NGF 28 kDa	5	4.9	0.01	0.055
BDNF	4	5	0.30	0.006

Discussion

Increases in two neurotrophins implicated in pain (NGF, BDNF) and disc pathology were detected in cervical spinal discs in association with prolonged and repeated exposure to WBV that produced sustained sensitivity (Figs. 2 & 3; Table 1). In fact, the approximate 5-fold changes in BDNF and the 28 kDa proNGF isoform were associated with a similar increase in behavioral sensitivity (Fig. 2; Table 1).

The 75 kDa high molecular weight form of NGF was increased by only 2-fold. Despite the large increase in the smaller molecular weight form of proNGF in WBV discs over sham, this increase did not reach significance (Table 1). Yet, this may be due to the small sample size (discs from only 5 rats) of available disc protein for that assay (Table 1). Additional studies with increased sample sizes in these groups will further elucidate the extent of these changes.

The role of neurotrophins in pain and disc degeneration remains unclear. High molecular weight forms of NGF have been suggested as having a high affinity for the p75 receptor and in the initiation of processes leading to neuron cell death.[9] Reports suggest that increased neurotrophic factors in degenerate and painful discs promote in-growth of nerves into the disc.[6] While the current study supports the emerging hypothesis that spinal vibration can lead to pain that may originate from the degenerating disc,[3] it does not provide a direct link between the WBV exposure, changes in the disc, and pain.

These results do provide encouraging data suggesting such a link between disc pathology and pain in the cervical spine. Future studies varying the duration and magnitude of WBV in the context of disc pathology and/or pain will provide more insight into this challenging problem.

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Acknowledgments

This work was supported by the Department of Defense (W81XWH-10-2-0140).

Abstract Presented at Northeast Bioengineering Conference in March, 2012.

BIOMECHANICAL EFFECTS OF WHOLE BODY VIBRATION ON SPINAL LIGAMENTS: A POTENTIAL MECHANISM OF TISSUE DAMAGE

Benjamin R. Freedman, Hassam A. Baig, Benjamin B. Guarino, Beth A. Winkelstein
Department of Bioengineering
University of Pennsylvania, Philadelphia, PA

Background

- Warfighters are subjected to whole body vibration (WBV) during the operation of tactical vehicles. This WBV exposure is believed to lead to a host of spinal pathologies, as well as chronic back and neck pain [1].
- There is increasing evidence that pain can result from altered mechanics of spinal tissues that can stimulate mechanotransductive pathways leading to nociceptive processes [2, 3].
- The spinal facet capsular ligament has been shown to be a source of pain when it undergoes non-physiological mechanical loading [2, 3]. This ligament is at risk for such motions during WBV (Fig 1) when it can undergo repeated tensile loading. Yet, biomechanical characterization of the facet capsular ligament has not been defined for WBV exposures.

Objective: To determine if facet capsular ligament tensile properties are mechanically altered after WBV compared to control specimens.

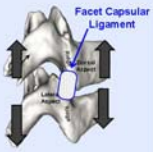

Fig 1. Anatomy & loading of facet capsular ligament during WBV.

Materials & Methods

Specimen Preparation:

- All experimental procedures were IACUC-approved.
- Thoracic spines were harvested *en bloc* from rats that had undergone whole body vibration (WBV; n=3) or were age-matched (Control; n=4). The WBV exposure was imposed daily for 7 days for 30 minutes at 15 Hz over a 1.5 mm distance under anesthesia. Spines were harvested at day 14, after rats were allowed a rest period of 7 days following WBV.
- T3/T4 and T5/T6 motion segments were dissected. The surrounding muscles were removed, and the interspinous ligament and ligamentum flavum were transected [2,3] (Fig 2A). The left facet capsular ligament was isolated for testing.

Mechanical Testing & Data Analysis:

- Specimens were mounted in a testing machine (Instron) to undergo tensile subfailure and failure loading (Fig 2).
- Force and displacement data were acquired at 1 kHz using Instron's Bluehill Software. The testing protocol included preconditioning, holds, and a subfailure distraction, followed by a ramp to failure (Table 1).
- MATLAB code was used to compute stiffness, yield, and failure loads and displacements. Separate Student's t-tests compared WBV and Control groups.

Table 1. Loading protocol under displacement control.

Block	Description
Preconditioning	N= 30, 0.1 mm
Hold	0 mm, 2 min
Subfailure Distraction	0.5 mm, 4 min
Hold	0 mm, 4 min
Failure	7 mm, 0.08 mm/s

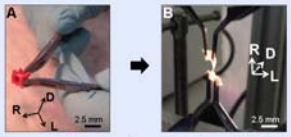

Fig 2. Axis labels: R-rostral, D-dorsal, L-left. **(A)** Motion segment prior to separation of the left capsular ligament. **(B)** Facet capsular ligament after failure.

Results

- Responses between Control and WBV groups were similar during tensile loading (Table 2), with both exhibiting a typical load-displacement curve (Fig 3).
- The tensile stiffness of the thoracic facet capsular ligament following WBV exposure was 70% greater ($p<0.01$) than the Control specimens (Table 2; Fig 4).
- Displacement and loads at both yield and failure were not different between groups (Table 2).

Fig 3. Representative load-displacement response for a Control thoracic facet capsular ligament.

Table 2. Summary of mechanical data for Control & WBV.

Parameter	Group: Control	Group: WBV
Stiffness (N/mm)*	2.32 ± 0.66	3.97 ± 1.14
Yield (N)	1.20 ± 0.34	1.21 ± 0.36
Yield Disp. (mm)	1.70 ± 0.58	1.71 ± 0.66
Gross Failure (N)	1.70 ± 0.48	1.76 ± 0.31
Gross Failure Disp. (mm)	2.32 ± 0.73	2.12 ± 0.82

* indicates $p<0.01$

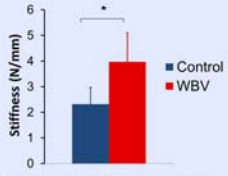

Fig 4. Stiffness was increased (*$p<0.01$) after WBV. Error bars are SD.

Discussion

- This study provides the first investigation of facet capsular ligament mechanics following WBV. Despite such repeated loading to the spine, tensile stiffness is the only mechanical parameter that appears to be altered from control responses (Table 2; Fig 4). Increased ligament stiffness may help stabilize the facet joint, but may also indicate early modifications in the health of the spine and its components.
- The increased stiffness in WBV (Table 2; Fig 4) may be due to increased collagen-1 deposition and cross-linking [4]. Investigating collagen organization and composition in these tissues would help elucidate these and other physiologic responses.
- Increased stiffness in the vibrated ligaments in this study is greater than reported in a study using rat flexor carpi ulnaris tendon vibrated 5 days a week for 5 weeks at 0.3 G 30 Hz [5]. However, differences in the tissue type, anatomy and loading protocol between these studies may explain this mismatch in findings. Additional studies with our WBV model are needed to further define the relationships between vibration exposure (magnitude, frequency, duration, etc.) and tissue responses.
- The lack of difference in the failure properties between groups (Table 2) may indicate that the thoracic facet joint does not undergo injurious loading during WBV. However, such mechanical changes may be more pronounced in other spinal regions that were not evaluated in the current study, such as the cervical and/or lumbar regions.

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Acknowledgment

This work is supported by a grant from the DOD (#W81XWH-10-2-0140).

A NEW MODEL OF REPEATED WHOLE BODY VIBRATION EXPOSURE IN THE RAT: BIOMECHANICAL & PAIN RESPONSES

BB Guarino[1], HA Baig[1], NV Jaumard[1,2], JL Branconi[1], DB Dorman[3], BL Shivers[3], BA Winkelstein[1,2]
Departments of [1]Bioengineering & [2]Neurosurgery, University of Pennsylvania, Philadelphia, PA
[3]Injury Biomechanics Branch, USAARL, Fort Rucker, AL

Background

- Vibration exposure has been linked to chronic pain and low back pain symptoms [1]. Several studies have investigated the mechanical effects of whole body vibration (WBV) using human and primate subjects [2,3].
- Limited studies have investigated the neurophysiologic effects of WBV exposure [4,5]. Although changes in several neuropeptides related to nociception (substance P and VIP) have been observed [4], no studies have directly assessed pain symptoms.
- The rat provides a desirable model system for studying pain and neurophysiology responses related to tissue loading and injury [6]. Yet, there is still very little known about its mechanical response to vibration. Although the dynamic response of other species has been defined [3,4], the transmissibility of the rat's spinal column and/or its response to whole body vibration along its long axis is unknown.

Study Objective: To characterize the frequency response of the rat during whole body vibration (WBV) and to investigate the effect of WBV on pain. To do this, two studies were performed: (1) a transmissibility study and (2) pain study.

Materials & Methods

General Procedures: Male Holtzman rats (n=27), weighing 215-350 grams, were used in experimental procedures that were approved by the University of Pennsylvania's IACUC and ACURO. For the transmissibility study, rats (n=10) were exposed to incremental vibration frequencies ranging from 3-15 Hz, and the transmissibility and resonance frequency were measured. For the pain study, separate groups of rats were exposed to 30 minutes of WBV daily at 5 Hz (n=7) or 15 Hz (n=7) for 7 days. An additional group of rats (n=4) underwent daily anesthesia exposure under the same protocol to serve as a sham control group. Pain responses were measured during and after the exposure period was terminated.

WBV Exposure & Biomechanical Measurements: Rats underwent WBV in the prone position, with the direction of vibration along the spine's long-axis, and were secured to an acrylic base plate using two velcro straps (Fig. 1). The base plate was coupled to a linear servo motor and driver (B2850, & VX5060H; Parker Hannifin) that imposed a 1.5 mm peak-to-peak displacement of the plate. Separate DC accelerometers (±50 Gs; sensitivity of 10 mV/G; Model 3521A; Dytran) were mounted on the base plate and affixed to the lumbar region of the rat by a strap (Fig. 1). Markers were placed on the base plate and the lumbar accelerometer and tracked by a high-speed CCD camera (IDS-UEYECP-1-1024SM; Phantom; 640S-960).

Vibration was performed under inhalation anesthesia (3.0-4% isoflurane) (Fig. 2). For the transmissibility study, rats underwent 60 seconds of sinusoidal vibration, followed by a rest period of 120 seconds before being exposed to the next, higher frequency, at frequencies ranging from 3-15 Hz. Accelerometer and image data were recorded during each frequency exposure at a sampling rate of 120 Hz. For the pain study, WBV was applied daily for a period of 30 minutes on 7 consecutive days.

Fig. 1. Image of a rat on the base plate attached to the motor exposed with Velcro for these studies. The lumbar (spinal) accelerometer is also shown mounted to the rat.

Fig. 2. Image showing the WBV exposure test setup, with the anesthesia system and scavenger, the accelerometers, and the camera in the foreground.

Transmissibility Analysis: Transmissibility was computed using two methods: data from the (1) accelerometers and (2) image markers were used as the input and output accelerations of the system. The input acceleration was measured using the accelerometer mounted on the base plate; the output acceleration was taken from the accelerometer affixed to the rat (Fig. 1). Markers on the accelerometers were used to derive the corresponding input and output motions of the system. For all analyses, 30 seconds of data were used. The corresponding input and output displacements were doubly differentiated to derive the corresponding input and output accelerations. Both image and accelerometer acceleration data were filtered using a 5th order Butterworth bandwidth filter. Transmissibility of the system for each vibration condition was calculated as the ratio of the RMS of the output acceleration to the RMS of the input acceleration [4]. Accelerations were transformed into the frequency domain and the phase shift at each frequency was calculated as the difference in angle between the two accelerations.

Behavioral Analysis: Behavioral sensitivity was assessed by measuring mechanical hypersensitivity in both of the forepaws and hind paws [7]. Hypersensitivity was measured prior to (day 0) the first day of WBV exposure and daily on the morning following the prior day's WBV session. Response thresholds were also measured for an additional 7 days after the WBV or anesthesia (sham) exposure period was ended. For each session, the plantar surface of each paw was stimulated with a range of von Frey filaments (0.4-26 g) using customary methods [7]. The average threshold was taken as the threshold for each paw, day, and group. A repeated-measures ANOVA with post hoc Bonferroni compared response thresholds between groups over time.

Results

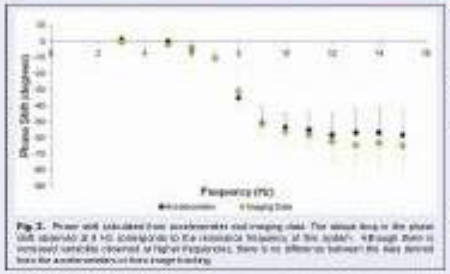

Fig. 3. Phase shift calculated from accelerometer and imaging data. The abrupt drop in the phase shift observed at 8 Hz corresponds to the resonance frequency of the system. Although there is increased variability observed at higher frequencies, there is no difference between the data derived from the accelerometers or from image tracking.

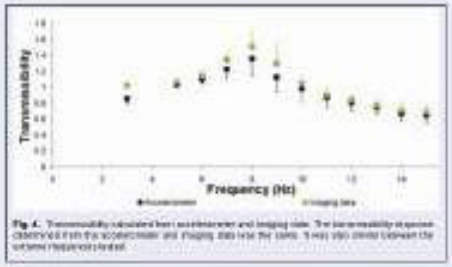

Fig. 4. Transmissibility calculated from accelerometer and imaging data. The transmissibility responses observed from the accelerometer and imaging data near the same. It was also similar between the extreme frequencies tested.

- The phase shift decreases with increasing input frequency, with an abrupt drop at 8 Hz (Fig. 3). Similarly, the peak transmissibility of a rat in this WBV system is also observed at 8 Hz (Fig. 4). The transmissibility response is similar at the extremes of frequencies tested: 0.9±0.1 at 3 Hz and 0.7±0.0 at 15 Hz (Fig. 4).
- The frequency responses are similar for those defined by the accelerometer and imaging data, respectively (Figs. 3 & 4).
- Behavioral sensitivity in both the forepaw and hind paw is induced immediately (at day 1) after a single WBV exposure, maintained during the WBV exposure period (days 1-6), and sustained for the 7 days after the termination of the WBV exposure (Fig. 5). There is no change in the sensitivity following sham procedures for either the forepaw or the hind paw (Fig. 5).
- The withdrawal threshold decreases significantly for both the 5 Hz ($p<0.001$) and 15 Hz ($p<0.001$) exposures (Fig. 5). However, there is no difference in sensitivity responses between these two exposure frequencies (Fig. 5).

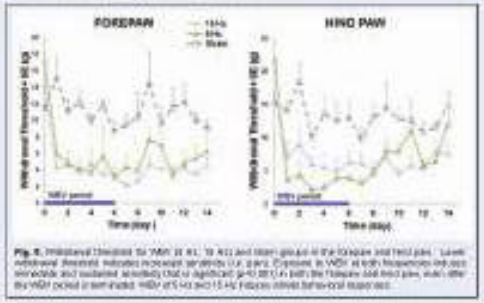

Fig. 5. Withdrawal thresholds for WBV at 5, 15 Hz and sham groups in the forepaw and hind paw. Lower withdrawal threshold indicates increased sensitivity (i.e. pain). Exposure to WBV at both frequencies induced immediate and sustained sensitivity that is significant ($p<0.001$) in both the forepaw and hind paw, even after the WBV period is terminated. WBV at 5 Hz and 15 Hz induces similar behavioral responses.

Discussion

The resonance frequency of the rat was found to be 8 Hz (Figs. 3 & 4), which is similar to other studies with different species [3,4,8]. The resonance frequency of both the human and rabbit has been approximated to be 4.5 Hz [4,8]; the primate has been shown to be in the range of 9–15 Hz [3]. For these studies, the primates and humans were vibrated in the seated position and transmissibility was measured along the axis of the spine [3,8]. The rabbit study was performed using a set-up similar to that used in the current study [4].

The transmissibility of the rat was found to be the same at the higher and lower ends of the frequencies tested here (Fig. 4) and may suggest that similar injuries may be induced regardless of vibration frequency. Yet, additional studies are needed to understand the relationship between the mechanical response of this system and the associated physiological correlates.

This study is the first to demonstrate that repeated WBV exposure, even for only 30 minutes a day, induces prolonged mechanical hypersensitivity in rats (Fig. 5). Although not previously shown, the behavioral responses of the current study are consistent with the observed changes in substance P and VIP in the lumbar dorsal root ganglia (DRG) in the rabbit after WBV [4]. Studies assaying neuroinflammatory responses in the muscle, disc, DRG, and spinal cord tissues in this model of vibration induced pain will provide insight about this type of injury and relate pain and nociceptive outcomes to exposure risks.

Although the applied acceleration of the rats during WBV in this study was aligned along the long-axis of the spine, this does not fully simulate a vertical spinal vibration that is common for human exposure. Of note, the acceleration magnitude was not held constant and it is not known how responses are changed for different acceleration profiles. In fact, an increase in acceleration has been shown to decrease the resonance frequency in human studies [2]. In contrast, the response at different accelerations was not different in a study of spinal vibration using a transducer that was directly attached to the spinous processes in the lumbar spine [8].

Continued studies under different vibration conditions and incorporating assays of tissue mechanics, physiology and function will improve our understanding of this system and its response to a common biomechanical injury mode.

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Acknowledgment

Grant support for this work provided by the Department of Defense (W81XWH-09-2-0183).